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PROGRAMA DE PÓS GRADUAÇÃO EM AGRONOMIA – PRODUÇÃO VEGETAL

Leonardo Deiss

SOIL ORGANIC PHOSPHORUS AND CARBON ON AGRICULTURAL AND NATURAL ECOSYSTEMS

Curitiba, 2016

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Tese apresentada ao Programa de Pós Graduação em Agronomia – Produção Vegetal, Departamento de Fitotecnia e Fitossanitarismo, Setor de Ciências Agrárias, Universidade Federal do Paraná, como parte das exigências para obtenção do título de Doutor em Ciências.

Comitê de orientação: Dr. Anibal de Moraes, Dr. Jeferson Dieckow e Dr. Alan J. Franzluebbers.

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PARECER

Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação em Agronomia - Produção Vegetal, reuniram-se para realizar a argüição da Tese de DOUTORADO, apresentada pelo candidato **LEONARDO DEISS**, sob o título **"SOIL ORGANIC PHOSPHORUS AND CARBON ON AGRICULTURAL AND NATURAL ECOSYSTEMS"**, para obtenção do grau de Doutor em Ciências do Programa de Pós-Graduação em Agronomia - Produção Vegetal do Setor de Ciências Agrárias da Universidade Federal do Paraná.

Após haver analisado o referido trabalho e argüido o candidato são de parecer pela "**APROVAÇÃO**" da Tese.

Curitiba, 24 de Outubro de 2016.

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FÓSFORO E CARBONO ORGÂNICO DO SOLO EM ECOSSISTEMAS AGRÍCOLAS E NATURAIS

RESUMO

A disponibilidade de solos e seus recursos está reduzindo com a evolução da humanidade e os impactos negativos, decorrentes do mau uso dos solos, estão afetando o desenvolvimento sustentável da agropecuária mundial. Portanto, se faz necessário o desenvolvimento de alternativas que permitam um uso mais sustentável dos solos, para atender as demandas no setor de produção agropecuária do século 21. O objetivo geral desta tese foi estudar solos de sistemas de produção agropecuária que buscam a intensificação de uso sustentável, ou de ecossistemas naturais, como uma etapa para melhor entender dinâmicas complexas de nutrientes. Foram estudados a composição de fósforo (P) no solo em sistemas integrados de produção agropecuária (SIPA) no subtrópico brasileiro, bem como em ecossistemas naturais em escala global e por fim, a dinâmica do carbono (C) orgânico em um sistema agroflorestal localizado na Carolina do Norte, Estados Unidos da América. O primeiro objetivo específico foi determinar a composição de P no solo de agroecossitemas com baixa e alta complexidade trófica. Especificamente, objetivou-se qualificar e quantificar os componentes orgânicos e inorgânicos de P usando extratos de NaOH-EDTA e espectroscopia de ressonância magnética nuclear, para posteriormente caracterizar a ciclagem de P em resposta ao aumento da complexidade trófica com SIPA no subtrópico brasileiro. A presença do pastejo resultou em maior concentração de ortofosfato total e biodisponível (i.e., Mehlich-I) e também diminuiu a concentração de P orgânico, incluindo os inositol fosfatos. O pastejo aumentou a biodisponibilidade de P e reduziu a concentração de P orgânico recalcitrante (i.e., inositol fosfatos), portanto, concluiu-se que a integração entre lavoura e pecuária pode ser uma alternativa sustentável para aumentar o uso do P nos sistemas de produção no subtrópico brasileiro. O segundo objetivo específico foi analisar com meta-regressão (meta-análise), a dinâmica do P em diferentes texturas de solo e de clima em escala global,

relacionando os grupos funcionais de P com o pH, concentração de C, relação CN e relação CP do solo em ecossistemas naturais. A composição de P orgânico teve uma resposta complexa à estas características do solo. A relação de monoesteres para o P orgânico aumentou com o aumento do pH, e diminuiu com o decréscimo da concentração de C, relação CN e relação CP, sem haver resposta particular para os locais e textura do solo. Em contraste, a relação de diesteres para o P orgânico bem como a relação diesteres para monoesteres teve o comportamento inverso, diminuindo com o aumento do pH, e aumentando com o aumento da concentração de C, relação carbono-nitrogênio (CN) e relação carbono-fósforo (CP). Portanto, concluiu-se que o pH, a concentração de C e as relações CN e CP são importantes fatores na determinação das proporções dos grupos funcionais de P orgânico do solo. O terceiro objetivo específico foi determinar a distribuição espacial de atributos do solo (textura e frações de C orgânico do solo) usando a combinação de espectroscopia de reflectância no infravermelho proximal (NIRS) e geoestatística, em um experimento de sistema agroflorestal localizado na Carolina do Norte, Estados Unidos da América. O NIRS foi uma ferramenta útil para predizer a textura do solo e as frações de C do solo. Na fase de calibração e validação do NIRS, o modelo de máquina de vetores de suporte teve uma performance melhor do que o modelo de mínimos quadrados parciais na predição das características do solo. A geoestatística aumentou os erros em relação àquales obtidos somente com o NIRS. Entretanto, a geoestatística possibilitou realizar a exploração das características espaciais da textura do solo e frações de C. A combinação do NIRS com a geoestatística pode ser utilizada para avaliação de atributos do solo deste sistema agroflorestal e de outros sistemas de produção, permitindo assim aumentar a sustentabilidade dos agroecossistemas através do manejo com agricultura de precisão.

Palavras chave: integração lavoura-pecuária, plantio direto, ciclagem de nutrientes.

SOIL ORGANIC PHOSPHORUS AND CARBON ON AGRICULTURAL AND NATURAL ECOSYSTEMS

ABSTRACT

Soil resources are narrowing as human evolution occurs and the negative feedbacks resulting from soil misuse are affecting agriculture's sustainable development worldwide. Therefore, alternatives that allow a more sustainable use of soils are necessary, to fill demands of the 21-century agriculture. The general objective of this thesis was to evaluate soils of agricultural systems that pursue sustainable intensification and natural ecosystems as a step to understand complex nutrient dynamics, which knowledge might help to adapt management by agriculture. It was studied the soil phosphorus compounds on integrated crop-livestock systems in Subtropical Brazil and on natural ecosystems across the world and soil organic carbon (C) dynamics in an agroforestry system on a Coastal Plain in United States of America. The first specific objective was to determine soil P composition from agro-ecosystems with low and high trophic complexity. Specifically, we wanted to qualify and quantify soil organic and inorganic P fractions using NaOH-EDTA extraction and nuclear magnetic resonance spectroscopy, and characterize P cycling in response to increasing complexity with integrated crop-livestock systems in subtropical Brazil. Our results were that in these agro-ecosystems, grazing compared with no-grazing had greater soil P content as total and bioavailable (i.e., Mehlich-I) orthophosphate and lower soil organic P and fewer monoesters, including inositol phosphates. Grazing increased P bioavailability and reduced recalcitrant organic P (i.e., inositol phosphates) concentration in soil; therefore, we conclude that integrating crop and livestock systems can be a sustainable alternative to improve P use in farming systems of subtropical Brazil. The second specific objective was to analyze through meta-regression, soil organic phosphorus dynamics among different soil textures and locates at global scale, relating its organic functional groups with soil pH, C concentration, carbon-to-nitrogen (CN) ratio and carbon-to-phosphorus (CP) ratio on natural ecosystems. We found that

soil organic P composition had a complex response to those soil characteristics. Monoesters-to-organic P ratio increased as pH increased, and decreased as C concentration, CN ratio and CP ratio increased, with no particular response among locates and soil textures. In contrast, diesters-to-organic P ratio as well as diesters-to-monoesters ratio had the opposite behavior, decreasing its concentrations as pH increased, and increasing as soil C concentration, CN ratio and CP ratio increased. Therefore we concluded that soil pH, C concentration, CN ratio and CP ratio are important factors in determining proportions of soil organic P functional groups. The third specific objective was to determine the spatial distribution soil properties (soil texture and organic C fractions) using a combination of near infrared spectroscopy and geostatistics, in an emerging agroforestry system experiment on a Coastal Plain site in North Carolina. Near-infrared spectroscopy was a useful tool to predict soil texture and soil organic carbon (SOC) fractions. Using chemometrics to calibrate NIRS, a support vector machine model performed better than a partial least squares model to predict soil texture (sand and clay) and SOC fractions (total, particulate, and mineralizable C determined as the flush of CO_2-C following rewetting of dried soil). Geostatistics increased errors of soil properties compared to those obtained solely by NIRS prediction. Nonetheless, geostatistics was useful to explore spatial patterns of soil texture and SOC fractions. Combining NIRS and geostatistics can be promoted for soil evaluation of this agroforestry system and in other landscapes to increase sustainability of agroecosystems through field-specific precision management.

Key words: mixed crop-livestock, no-tillage, nutrient cycling.

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SUMMARY

1. INTRODUCTION

Soil resources are narrowing as human evolution occurs and negative feedback resulting from soil misuse is disturbing the world's sustainable development. Worldwide, land availability for agriculture is declining as urbanization, desertification and salinization rates increase as well as sea level rises (Fedoroff et al., 2010; Amundson et al., 2015). In addiction, pressure on preserving native forests and wetlands, which can act both as carbon sinks and preserve biodiversity is as well increasing (Lenart, 2009; IEG, 2011). However, policies seeking to preserve biodiversity and ecosystem services must indeed take into account the need to feed the human population (Godfray and Garnett, 2014).

Many soils currently in use are being degraded by wrong anthropic actions or indirectly as an environmental change consequence. Agriculture is using 11% of the world land surface and 25% is already in a highly degraded state (FAO, 2011). Soil degradation occurs by the imbalance between biochemical outputs and inputs as well as structural changes, being characterized by the inability or reduction on supporting life. It is being caused in agricultural fields by no fertility replenishment and huge amounts of soil lost by erosion. Furthermore, low quality or mismanaged soils are responsible for increased levels of land, water and air pollution (e.g., heavy metals, eutrophication and greenhouse gases). Improving soil organic carbon concentration in carbon-depleted soils is a potential path towards mitigation of soil erosion, water quality deterioration, biodiversity loss, and greenhouse gas emissions.

Climate is changing in an extent that its extreme consequences are being uncontrollable. Those consequences are affecting agricultural lands by increasing temperature, droughts and floods, and loss of biodiversity (Lichtfouse et al., 2009; Fedoroff et al., 2010). The global food system needs to "mitigate greenhouse gas emissions while simultaneously adapting to its consequences" (Godfray and Garnett, 2014). According to Lal (2014), soil is the largest potential pool for carbon storage to mitigate aggravating climate adversities. Therefore, stabilizing and increasing soil organic carbon content can help diminish climate change and its impacts on ecosystems.

While biodiversity is declining in the planet the human population does not stop growing. The stabilization on population growth is unlikely to happen in this century and

population is expected to be between 9.6 and 12.3 billion in 2100 (Gerland et al., 2014). The sustainable development must end poverty and hunger as well as achieve food security and improved nutrition (UN-SDSN, 2015). Proper nutrition contributes to human development, but we are still having over one in nine people undernourished (FAO, IFAD and WFP, 2015). FAO, IFAD and WFP (2015) suggest that progress towards food security can be achieved with economic growth, agricultural productivity growth and social protection.

The world is facing a dichotomy between remediation of the negative climate-derived impacts promoted by agriculture (as well as many other branches of human society) versus sustaining development and increase of human population, which will increase in quality and quantity the demand for agricultural products. To achieve food security, food production must grow substantially while its environmental footprint must shrink dramatically (Foley et al., 2011). Therefore, emergent alternatives to fill demands of the 21-century agriculture are welcome and necessary.

Technological advance of agriculture may give more emphasis on how to sustainably increase the yield potential to fill the gaps in a broad sense, intensifying agricultural production in a safe and diversified way, concomitantly with rising good environmental aspects, such as soil organic carbon storage and resilience. According to Godfray and Garnett (2014), sustainable intensification must (i) increase food production meeting the food security, (ii) this increase must come mainly from existing agricultural land, (iii) increase the food production sustainability, and (iv) many tools and production methods must be considered to achieve these goals.

Current barriers to achieve sustainable intensification are the limited reserves of nutrients (phosphorus, potash and calcium), the nitrogen fertilizers derived green house gasses emission, nitrogen and phosphorus pollutant potential to water sources, and soil carbon deficits (Foley et al., 2011; Godfray and Garnett, 2014; Amundson et al., 2015). Phosphorus, which is a key component of agricultural production, has become a global security issue due to rapidly dwindling and limited ore reserves. Inefficient use and recovery by animals and plants and high losses to the environment via erosion, surface runoff, and subsurface leaching in agricultural systems contribute to this reality (Elser and Bennett, 2011, Cordell and White, 2013). All of these issues are intertwined and

require strategies of sustainable intensification to somehow balance global demands from agriculture (Godfray and Garnett, 2014).

Alternatives to improve the processes on agriculture to achieve sustainable intensification and food security are integrated crop-livestock systems, conservation agriculture practices and precision agriculture (Komatsuzaki and Ohta, 2007; Gebbers and Adamchuk, 2010; Palm et al., 2014; Lemaire et al., 2014; Neufeldt et al., 2015). Integrated crop-livestock systems, which can be developed using a variety of approaches (Bell and Moore, 2012; Moraes et al., 2014; Sulc and Franzluebbers, 2014), provide an opportunity to intensify with sustainability, restoring the environmental quality and enhancing biodiversity of simplified and specialized agro-ecosystems. At the same time, the corresponding land use and its capacity must be considered while mitigating risk and improving soil and water preservation (Herrero et al., 2010; Moraine et al., 2016). Simultaneously, conservation practices (Palm et al., 2014) may be part of the agricultural design, in which must also be considered, climate-change resiliency through soil organic matter management, improved water harvest and conservation, and increased agrobiodiversity, (Altieri et al., 2015). Finally, agricultural productivity and environmental quality of fields and regions could become more balanced if geographic precision of soil properties is known, and subsequently managed appropriately. In this case, greater management precision could impact all system components, including crop and pasture yield, greenhouse gas emissions, water and soil quality, and economic viability.

The general objective of this thesis was to evaluate soils of agricultural systems that pursue sustainable intensification and natural systems as a step to understand complex dynamics of soil phosphorus and carbon, which knowledge might help to adapt management by agriculture. Soil phosphorus compounds on integrated crop-livestock systems in Subtropical Brazil and on natural ecosystems around the world and soil organic carbon dynamics in an agroforestry system on a Coastal Plain in United States of America were studied.

The first specific objective was to determine soil P composition from agro-ecosystems with low and high agricultural complexity (no-grazing versus grazing). Specifically, we wanted to (i) qualify and quantify soil organic and inorganic P fractions

using NaOH-EDTA extraction and nuclear magnetic resonance (NMR) spectroscopy and (ii) characterize soil P composition in response to increasing complexity with integrated crop-livestock systems (ICLS) in subtropical Brazil.

The second specific objective was to analyze through meta-regression, soil organic phosphorus dynamics among different soil textures and locates at global scale, relating its organic functional groups with soil pH, carbon (C) concentration, carbon-to-nitrogen (CN) ratio and carbon-to-phosphorus (CP) ratio on natural ecosystems.

The third specific objective was to determine the spatial distribution soil properties (soil texture, soil organic carbon, particulate organic carbon and mineralizable C) using a combination of near infrared spectroscopy and geostatistics, in an emerging agroforestry system experiment on a Coastal Plain site in North Carolina.

CHAPTER 1

Soil phosphorus compounds in integrated crop-livestock systems of subtropical Brazil[1]

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Soil phosphorus compounds in integrated crop-livestock systems of subtropical Brazil

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ABSTRACT

Soil phosphorus (P) utilization may be affected by agricultural complexity, in particular when combining annual crops and livestock grazing on the same land area and at overlapping times. Our objectives were to qualify and quantify soil organic and inorganic P compounds using sodium hydroxide-ethylenediaminetetraacetic acid (NaOH-EDTA) extraction and ^{31}P nuclear magnetic resonance spectroscopy (^{31}P NMR) in response to increasing complexity with integrated crop-livestock systems (ICLS) in subtropical Brazil. Soil at a depth of 0–5 cm was collected from three long-term (7 to 12 years) cropping studies with and without ruminant grazing of cover crops. All sites were managed under no tillage, and treatments with livestock were managed with moderate grazing intensity. In these agro-ecosystems, grazing compared with no-grazing had greater soil P content as total and bioavailable orthophosphate and lower soil organic P and fewer monoesters, including inositol phosphates. Grazing increased P bioavailability and reduced recalcitrant organic P concentration in soil; therefore, cropping systems that integrate livestock (ICLS) can be a sustainable alternative to improve P use in farming systems of subtropical Brazil.

1. Introduction

Phosphorus (P) is a key component of agricultural production and has become a global security issue due to rapidly dwindling and limited ore reserves amidst a rapidly expanding human population. Inefficient use and recovery by animals and plants and high losses to the environment in agricultural systems contribute to this reality (Elser and Bennett, 2011; Cordell and White, 2013). These issues are intertwined and require strategies of sustainable intensification to somehow balance demands (Godfray and Garnett, 2014).

One approach to sustainably intensify agriculture and increase P cycling may be through integrated crop-livestock systems (ICLS), which can be developed using a variety of approaches (Bell and Moore, 2012; Moraes et al., 2014; Sulc and Franzluebbers, 2014). In subtropical Brazil (south of 23° S), agriculture is purposely intensified with moderate stocking of livestock grazing on annual and perennial forages in crop sequences under no-tillage. Ruminant livestock can alter agro-ecosystem properties through their impacts on forage and soil characteristics via spatial and temporal distribution of grazing (i.e. consumption of forage), trafficking, and excretion. Litter decomposition can be accelerated via exposure to rumen microbes (Haynes and Williams, 1993; Acosta-Martínez et al., 2010a, 2010b; Davinic et al., 2013).

In the soil, P is distributed into organic and inorganic forms and these are subdivided into functional groups. The functional groups of organic P are orthophosphate monoester, orthophosphate diester, phosphonate and polyphosphate (Condron et al., 2005) and of inorganic P are orthophosphate, polyphosphate and pyrophosphate (Cade-Menun and Preston, 1996). To break down diesters, plants and microorganisms require hydrolysis by both phosphodiesterases and phosphomonoesterases to release free phosphate, whereas monoesters require only the latter (Turner, 2008a). However, although inositol phosphates are classified as monoesters, they require both solubilization and hydrolysis by phytase to release a free phosphate and they can be strongly bound to metal oxides, clays, and organic matter (Turner, 2008a).

Inorganic P in soils can be sorbed to clays and Al and Fe oxyhydroxides, which depends on sorption and desorption processes to release orthophosphate to soil solution. Moreover, inorganic P can also be present as secondary (e.g., Ca, Fe and Al phosphates) and

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primary P minerals (e.g., apatites), depending on dissolution processes to release orthophosphate, and precipitation as a way to fix P in the case of secondary minerals (Shen et al., 2011).

We hypothesized that livestock grazing with ICLS would enable greater P utilization due to the cycling of P in the soil-plant-animal continuum, thereby increasing availability of P in soil.

Our objective was to determine soil P composition from agro-ecosystems with and without livestock grazing. Specifically, we wanted to qualify and quantify soil organic and inorganic P compounds using NaOH-EDTA extraction and nuclear magnetic resonance (NMR) spectroscopy to characterize soil P composition in response to increasing agricultural complexity with ICLS in subtropical Brazil.

2. Materials and methods

2.1. Study sites

Three long-term experiments (7 to 12 years) investigating various aspects of ICLS were established in Rio Grande do Sul and Parana states of southern Brazil (Table 1). Each experiment had treatments selected to compare low and high agricultural complexity, i.e. without and with grazing of cover crops. Baseline cropping system was summer grain crop followed by winter cover crop at all locations. The same cover crop was used whether grazed or not. All experiments used no-till soil management, and moderate grazing intensity if livestock was part of the treatment. When grazed, a target forage height was achieved using a put-and-take stocking technique (Mott and Lucas, 1952). At each location, each treatment was sampled three times. All grazed experimental units (~0.2 ha for sheep and ~2.5 ha for cattle) had at least three plots within a paddock for continuous stocking. Ungrazed plots were not always replicated in the design (i.e., São Miguel das Missões and Eldorado do Sul), but plots were subdivided for sampling purposes.

Table 1
Climate and agronomic history of experiments at Guarapuava, São Miguel das Missões and Eldorado do Sul with no-grazing (sole-cropping system) or grazing of winter cover crops as an integrated crop-livestock system in subtropical Brazil.

Sites	Guarapuava	São Miguel das Missões	Eldorado do Sul
Coordinates	25° 33′ S 51° 29′ W	29° 03′ S 53° 50′ W	30° 05′ S 51° 39′ W
Climate (Köppen system)[a]	Cfb	Cfa	Cfa
Altitude above sea level (m)	1100	465	46
Soil classification[b]	Oxisol	Oxisol	Ultisol
Clay content (%)	61.4[c]	54.0[d]	15.5[e]
Mean precipitation (mm year^{-1})	1900	1850	1440
Mean annual temperature (°C)	17.5	19.0	19.6
Experiment establishment (year)	2006	2001	2003
Fertilizer input (kg ha^{-1} year^{-1})[f]			
N	285	56	161
P	70	9	27
K	170	24	47
Grazing P output: crops/animals (kg ha^{-1} year^{-1})	34.5/3.2	12.8/3.9	13.1/3.4
No-grazing P output: crops (kg ha^{-1} year^{-1})	32.6	13.4	11.4
Grazing P budget (kg P ha^{-1})	32.1	−8.0	10.5
No-grazing P budget (kg P ha^{-1})	37.2	−4.7	15.6

[a] Cfa: subtropical with humid and hot summer and Cfb: temperate with no definite dry season.
[b] Soil Survey Staff (2010).
[c] Sandini et al. (2011).
[d] Costa et al. (2014).
[e] Barth-Neto et al. (2014).
[f] Since the experiment establishment.

The field trial at São Miguel das Missões was the oldest. Prior to the current experiment that started in 2001, land was in no-till soybean (*Glycine* max) in summer rotated with *Avena strigosa* for seed production in winter. This experiment had four cattle grazing intensities of the winter forage, *Lolium multiflorum* and *A. strigosa* mixture (forage maintained at 10, 20, 30, or 40 cm height). Soybean was grown in summer. The moderate grazing intensity (20-cm height) had adequate grazing intensity to promote sustainability of ICLS (Chávez et al., 2011; Assmann et al., 2014; Costa et al., 2014; Martins et al., 2014a, 2014b), and therefore, was selected to compare with an ungrazed soybean/cover crop mixture. Grazing was with 1-year-old beef calves starting in mid-July and ending in early November. On average, four animals ha^{-1} were needed to maintain a sward height of 20 cm. Soybean was seeded between late November and early December. Nitrogen fertilization as urea (46% N) was applied at 45 to 90 kg N ha^{-1} (lower level during first 10 years) approximately 45 days after cover crop seeding (Anghinoni et al., 2013). Soybean was fertilized with an average of 3 kg N ha^{-1}, 27 kg P ha^{-1} and 69 kg K ha^{-1} to achieve a target of 4.0 Mg ha^{-1} yield, as recommended by the Local Soil Fertility Committee (CQFS-RS/SC, 2004). Lime (62% calcium carbonate equivalent) was applied to the surface of the entire area at 4.5 Mg ha^{-1} in November 2001 (Martins et al., 2014a, 2014b).

The experiment at Eldorado do Sul was initiated in 2003 comparing continuous and rotational stocking of sheep under low and moderate grazing intensity (i.e. 5.0 and 2.5% consumption, NRC, 1985), as well as an ungrazed winter cover crop of *L. multiflorum*. Soybean only and soybean-maize (*Zea mays*) were grown in the summer. From 1999 to 2003, land was under no-till production of *Pennisetum glaucum* in summer and *L. multiflorum* in winter (Carassai et al., 2011). We selected the continuous, moderate grazing intensity of winter cover crop in soybean-maize rotation and no grazing of the same crop sequence as treatments for analysis in this study. Mean sward height of the grazed treatment was 22.5 cm. The previous crop prior to soil sampling was soybean. Texel and Ile de France cross-bred lambs (initial average weight of 21.9 kg) or single-bearing lactating ewes (initial average weight of 63.5 kg) and lambs (initial average weight of 9.6 kg) of the same breed were used for grazing for approximately 120 days (for more details see Barth-Neto et al., 2014).

The experiment in Guarapuava was established in 2006. Prior to that time, the land was managed for 8 years with no-till summer crops. The experimental design was four N fertilizer levels (0, 75, 150, and 225 kg N ha^{-1}) applied to a winter cover crop mixture (*L. multiflorum* + *A. strigosa*), which was either grazed by sheep or not grazed. Summer cropping was a common bean (*Phaseolus vulgaris*)-maize rotation (fertilized with 120 and 150 kg N ha^{-1}, respectively). We selected an intermediate winter N level (150 kg N ha^{-1}) for both grazed and ungrazed cover crops, which was considered adequate to optimize a residual N effect for rotated crops in ICLS (Sandini et al., 2011). The previous crop before soil sampling was common bean. At least six tester lambs (2 months old, initial average weight of 25 kg) and a variable number of put-and-take lambs were stocked to maintain sward height of ~15 cm. Since experimental initiation, lime was only applied in April 2013 at a rate of 4.0 Mg ha^{-1}. Liming was performed according to the Local Soil Fertility Committee (CQFS-RS/SC, 2004).

The P budget was determined according to the balance method proposed by Syers et al. (2008), which considered the yield and P uptake relative to the amount of P applied. Crop and animal yield data were collected from Martins et al. (2015) for São Miguel das Missões, from Sandini (2009); Pellegrini et al. (2010); Sandini et al. (2011); Sartor (2012), Andreolla (2010), and Andreolla et al. (2014) for Guarapuava, and Lunardi et al. (2008); Barth-Neto (2015), and Macari et al. (2011) for Eldorado do Sul. To determine P uptake relative to crop yield, 5 kg P Mg^{-1} grain yield for soybean (Borkert et al., 1994) and 4.67 kg P Mg^{-1} grain yield for common bean (Rosolem and

Marubayashi, 1994) were considered. For maize, we used the equation $y = 4.98x - 9.06$, where y is the P uptake in kg P and x is the maize grain yield in Mg^{-1} (Coelho and França, 1995). To determine animal P uptake, 7.1 g P kg^{-1} live weight was used for bovine (Hattey et al., 2003) and 6.0 g P kg^{-1} live weight was used for ovine (Bellof et al., 2006).

2.2. Soil sampling

Soil was sampled between late August and early October in 2013 during growth of winter cover crops. Specifically, soil at depth of 0–5 cm was collected on August 21 at São Miguel das Missões, September 17 at Eldorado do Sul, and October 7 at Guarapuava. Samples consisted of approximately 20 cores ha^{-1}, randomly taken with an auger (5 cm-diameter), and composited per experimental unit. Since ungrazed and sheep-grazed experimental areas were smaller than areas grazed by cattle, a reduced number of soil cores was sampled with a minimum of 10 cores per experimental unit. Where animals were present, locations near fences (≤10 m) and recently deposited feces (≤40 cm) were avoided. Soil was sieved (<8 mm) at field-moisture content for the NaOH-EDTA extraction, and afterwards, was dried (50 °C, 5 days) for all other analyses.

2.3. Laboratory analyses

For the analysis of total elemental P, Al, Fe, Mn, C and N, as well as amorphous P, Fe, and Al oxides, about 20 g of soil was crushed in a mortar to pass a 0.25-mm mesh.

2.3.1. Total elemental P, Al, Fe, and Mn

A 0.25 g subsample was digested in a microwave oven (Milestone Ethon Plus) with 4 mL of concentrated HNO_3, 3 mL of HF, and 1 mL of 30% H_2O_2 (v/v) (adapted from Lim and Jackson, 1986). The flask was heated for 10 min at 1000 W until reaching 200 ± 2 °C and maintained for ~20 min. After 30 min of cooling inside the oven, the suspension was filtered to eliminate possible refractory residual material (e.g., titanium oxides). The P, Al, Fe, and Mn concentrations were determined in the solution by inductively-coupled plasma optical-emission spectrometry (ICP-AES).

2.3.2. Total elemental C and N

A 25 mg subsample was subjected to combustion with Vario EL III elemental analyzer (Elementar Analysen systeme GmbH) for determination of total C and total N.

2.3.3. Amorphous P, Fe, and Al oxides, P saturation and Mehlich-I P

A 0.25 g subsample was extracted with 0.2 M ammonium oxalate (AO) at pH 3.0 in the dark (McKeague, 1978) to determine amorphous P, Fe, and Al oxides ($_{ox}$). Concentrations of P_{ox}, Al_{ox}, and Fe_{ox} were determined in the extracts by ICP-AES.

According to van der Zee and Van Riemsdijk (1988), the degree of P saturation was determined as:

$$DPS = 100 \times P_{ox}/[\alpha\,(Al_{ox} + Fe_{ox})]$$

where P_{ox}, Al_{ox} and Fe_{ox} were expressed in mole element per soil unit mass and α was an empirical factor ($\alpha = 1$).

To extract P with Mehlich-I, soil was sieved to pass through a 2 mm screen and a 10 g subsample was extracted with 100 mL of 0.05 M HCl and 0.0125 M H_2SO_4. Samples were shaken for 10 min and then rested for 24 h. A 5 mL aliquot of the supernatant was mixed with amonium molybdate and ascorbic acid. P in solution was determined with a spectrophotometer and readings were conducted at 660 nm wavelength.

To determine the molybdate-reactive P concentrations, 1 g soil was extracted for 16 h with 50 mL 0.5 M H_2SO_4 (O'Halloran and Cade-Menum, 2008). According to O'Halloran and Cade-Menum (2008), P in solution was determined with a spectrophotometer and readings were conducted at 820 nm wavelength. Molybdate-unreactive P was determined by the difference between total P and molybdate-reactive P concentrations.

2.3.4. NaOH-EDTA extraction and NMR spectroscopy

Due to the expense of analysis, we composited the three replicates from each treatment and site to obtain a total of 6 samples for NaOH-EDTA extraction (adapted from Turner, 2008b). Using coarsely sieved (8 mm) fresh soil (Condron and Newman, 2011), 1.50 ± 0.01 g soil was weighed into a 50 mL centrifuge tube. A separate subsample was used for determining moisture content, weighing before and after drying soil in an oven (65 °C until constant weight). A 30-mL solution containing 0.25 M NaOH and 0.05 M Na_2EDTA [i.e., a 1:20 solid to solution ratio (Turner, 2008b)] was added to the soil in a centrifuge tube and shaken for 16 h at ambient laboratory temperature (~22 °C) (Turner and Blackwell, 2013; Ahlgren et al., 2013). The suspension was centrifuged at 3600*g* for 30 min and the supernatant filtered through a qualitative filter paper (pore size: 11 μm) (Turner, 2008b).

A1-mL aliquot of extract was collected and neutralized using phenolphthalein indicator and 3 M H_2SO_4 and diluted to 20 mL with deionized water (Turner and Blackwell, 2013) for determining total P, Fe, and Mn concentrations by ICP-OES. A 20-mL aliquot (Turner, 2008a) of extract was frozen at −30 °C for 3 days and subsequently lyophilized by freeze-drying at −50 °C for 5 days. The resultant powder was homogenized and approximately 400 mg was re-dissolved in an Eppendorf vial with 1.0 mL of a solution containing 0.25 M NaOH, 0.1 M Na_2EDTA (Turner, 2008b), and 1.0 mL of deuterium oxide (D_2O) for signal lock. Solution was vortexed for 5 min and then 1.0 mL of supernatant transferred to a 5-mm diameter NMR tube, to which the internal standard MDPA (Methylenediphosphonic acid ≥99%, Sigma-Aldrich®) was added for analyses by solution ^{31}P NMR spectroscopy. The amount of MDPA added to the NMR tube was equivalent to 0.0215 μg P for Eldorado do Sul and 0.043 μg P for the two other locations.

Experimental parameters used for NMR are described in the following. The spin-lattice relaxation time (*T*1) was obtained indirectly from the P to paramagnetics ratio in NaOH-EDTA extract, according to McDowell et al. (2006) and Cade-Menun and Liu (2013). The highest P:(Fe + Mn) [w/v] ratio was for orthophosphate at Guarapuava, from which the delay time was determined as 3.6 s with 0.4 s acquisition time. Other parameters used were proton decoupling and 90° pulse. 15,000 scans were obtained with sample temperature regulated at 25 °C, using a Bruker AscendTM 600 MHz spectrometer equipped with a 5-mm QXI probe (sensitive for 1H and 31P).

Phosphorus concentrations were quantified using the internal standard MDPA as reference. Peak areas were calculated by integration of spectra using the peak picking component and processed with 3.0 Hz line-broadening of TopSpin 3.2 software (Bruker Biospin GmbH, Rheinstetten, Germany). Peaks were identified based on their chemical shifts (ppm) according to Doolette et al. (2009) and Turner et al. (2003). The range of chemical shifts were 16.86 ppm for MDPA, 5.68 to 5.72 ppm for orthophosphate, −4.85 to −4.79 ppm for pyrophosphate, 5.33 to 5.43 ppm, 4.41 to 4.51 ppm, 4.05 to 4.12 ppm and 3.88 to 3.96 ppm for myo-Inositol hexakisphosphate, 3.58 to 3.60 ppm for scyllo-Inositol hexakisphosphate, 5.15 to 5.16 ppm for D-glucose-6-phosphate, 4.66 to 4.69 ppm for α-glycerophosphate, 4.27 to 4.37 ppm for β-glycerophosphate, 4.17 to 4.24 ppm for adenosine 5′ monophosphate, −0.71 to −0.73 ppm for diester, and between 5.7 ppm and 3.9 ppm for other monoesters, excluding identified peaks. According to Young et al. (2013) and Cade-Menun et al. (2010), organic P functional groups were determined by reconstituting values of diesters converted to monoesters. This was done by adding to the total diesters concentration, the α- and β-glycerophosphate concentrations, which resulted in the reconstituted diesters

concentration denominated here as corrected diesters (cDiesters). As well, corrected monoesters (cMonoesters) concentration was given by subtracting from the total monoesters concentration, the α- and β-glycerophosphate concentrations. Total inorganic P was from the sum of NaOH EDTA extractable inorganic orthophosphate and pyrophosphate, while total organic P was the sum of NaOH EDTA extractable orthophosphate monoesters and diesters.

2.4. Statistical analyses

Statistical analyses were made using R Version 3.1.0 (© 2014 The R Foundation for Statistical Computing) with RStudio Version 0.98.942 (©2009–2013 RStudio, Inc). To compare agricultural complexity levels among the experiments, the statistical models considered two treatments (no-grazing and grazing) as a fixed variable and location as a random variable, using lme4-package. Means that differed significantly ($p < 0.1$) were separated with Tukey Contrasts, using doBy- and multcomp-packages. The agricultural complexity effect for individual locations was determined only for general soil characteristics. NMR variables did not have replicates within a location, and therefore, the two treatments (no-grazing and grazing) were analyzed across the three locations, which served as replicates using stats-package.

3. Results

Across all three experimental locations, soil C ($p < 0.05$) and N ($p < 0.01$) were lower with grazing, while Mehlich-I P concentration was greater with grazing than without grazing (Table 2). Grazing compared to no grazing increased Mehlich-I P concentration by 32%. Within each location, agricultural complexity had an effect on a few variables. At São Miguel das Missões, soil pH was greater with grazing than without grazing ($p < 0.05$). At Eldorado do Sul, grazing increased soil total P ($p < 0.01$), Mehlich-I P ($p < 0.05$), molybdate-reactive P ($p < 0.01$), C:P ratio ($p < 0.1$), N:P ratio ($p < 0.1$), P_{ox} ($p < 0.01$), Al_{ox} ($p < 0.01$) and Fe_{ox} ($p < 0.1$).

At all three locations, grazing resulted in lower P budget (i.e. more P taken off) than without grazing (Table 1). This result was mainly from the additional output from animal production. However at Guarapuava and Eldorado do Sul, the ICLS system (i.e. with grazing) had greater crop-derived P output from greater crop yield than without grazing. At Guarapuava, mean maize and common bean grain yields were 10.7 and 3.7 Mg ha^{-1} without grazing and 11.8 and 3.5 Mg ha^{-1} with grazing, respectively. At São Miguel das Missões, mean soybean grain yield was 2.7 and 2.6 Mg ha^{-1} without and with grazing, respectively. At Eldorado do Sul, mean maize and soybean grain yields were 3.3 and 1.3 Mg ha^{-1} without grazing and 4.0 and 1.2 Mg ha^{-1} with grazing, respectively. Annual animal production at São Miguel das Missões (bovine), Guarapuava (ovine) and Eldorado do Sul (ovine) was 550, 537 and 560 kg ha^{-1}, respectively.

The following soil P results were determined from spectra in Fig. 1 and represent means across the three locations. Soil inorganic P as % of total P basis was greater with grazing than without ($p < 0.05$), but there was no difference when expressed on mg kg^{-1} basis. Organic P was 18% lower (on mg kg^{-1} basis) with grazing than without (Table 3).

Soil organic P as corrected monoesters (cMonoesters) was 21% lower with grazing than without (mg kg^{-1} basis) (Table 3). Corrected diesters (cDiesters), cMonoesters to cDiesters ratio, and degradation peaks were not affected by agricultural complexity.

Several soil organic P compounds, including adenosine 5′ monophosphate, and myo- and scyllo-Inositol hexakisphosphate had lower concentration with than without grazing (Table 4). Grazing reduced soil inositol phosphate concentration by 25% (sum of myo- and scyllo-Inositol hexakisphosphate on mg kg^{-1} basis) compared with no grazing.

Regarding soil inorganic P compounds, total orthophosphate concentration was 8.4% greater with grazing (NaOH EDTA % basis) than without grazing. Pyrophosphate was not affected by agricultural complexity levels (Table 4).

4. Discussion

The three locations in this investigation represented a broad cross-section of southern Brazil with distinct characteristics of soil, climate and agronomic history. Despite these differences, key aspects of management were consistent, such as ruminants managed under moderate grazing intensity and soil managed under no-tillage. Moreover the soil sampling was conducted during the winter, when all locations were with C3 grasses with similar growth pattern. We compared the impact of grazing of cover crops in winter versus no-grazing (sole-cropping system), where the same crop sequences were maintained within each experiment (location). We selected treatments based on complexity (i.e. plant species diversity) existent in each experiment (location),

Table 2
Soil characteristics from Guarapuava, São Miguel das Missões, and Eldorado do Sul with no grazing (NG) (sole-cropping system) and grazing (G) of winter cover crops in an integrated crop-livestock system in subtropical Brazil.

	Guarapuava		São Miguel das Missões		Eldorado do Sul		Mean across locations	
Treatment	NG	G	NG	G	NG	G	NG	G
pH	6.4	6.4	5.4	5.2*[a]	6.2	6.2	6	5.9
Total C (g kg^{-1})	50	46	31	30	20	18	34	31*
Total N (g kg^{-1})	3.7	3.5	2.8	2.7	1.9	1.7	2.8	2.6**
Total P (g kg^{-1})	3.94	3.91	1.8	1.74	1.04	1.23**	2.3	2.3
Al (g kg^{-1})	55.8	60.6	30.8	32.5	25.5	25	37.4	39.4
Fe (g kg^{-1})	76.8	78.3	89.1	85.4	8.5	8.4	58.1	57.4
Mn (mg kg^{-1})	330	338	620	583	128	130	359	350
C:N ratio	13.1	13.3	10.9	10.7	10.4	10.7	11.5	11.6
C:P ratio	11.7	12.6	17.1	17	14.7	19.3″	14.5	16.3
N:P ratio	0.89	0.95	1.57	1.58	1.41	1.8″	1.3	1.4
P_{ox} (mg kg^{-1})	667	642	428	449	494	652**	530	581
Al_{ox} (g kg^{-1})	2	2	1.6	1.6	0.8	0.9**	1.5	1.5
Fe_{ox} (g kg^{-1})	2	2	2.5	2.3	0.9	1.2″	1.8	1.8
DPS % ($\alpha = 1.0$)	17.1	16.2	10.5	11.4	28.1	31	18.6	19.5
NAOH EDTA extracted P (%)	63.6	59.8	85.9	87.7	80	84.4	76.5	77.3
P (Mehlich I) (mg kg^{-1})	21.9	25.7	22.7	28.6	50.3	71.2*	31.6	41.8″
MRP (mg kg^{-1})	2370	2289	1061	1076	740	917**	1391	1428
MUP (mg kg^{-1})	1570	1625	739	663	303	313	871	867

DPS: Degree of phosphorus saturation (oxalate P/[α x (oxalate Al + Fe)] × 100). $_{ox}$: oxides. NAOH EDTA extracted P: NaOH EDTA P to total P ratio. MRP: Molybdate-reactive P. MUP: Molybdate-unreactive P.

[a] Significance codes between agricultural complexity levels: ***<0.001, **$p < 0.01$, *$p < 0.05$ and ″$p < 0.1$.

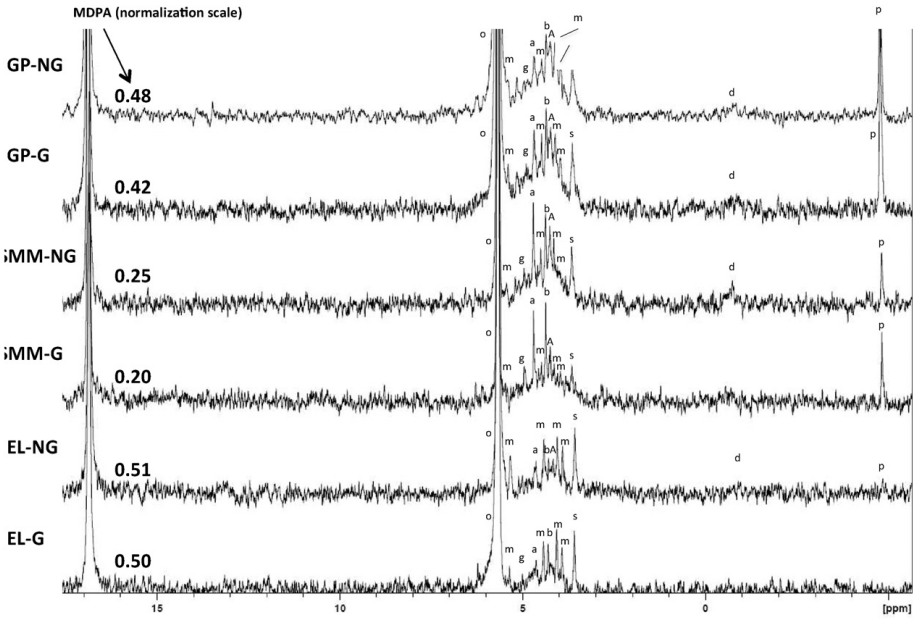

Fig. 1. ^{31}P nuclear magnetic resonance spectra of soil NaOH EDTA extracts from Eldorado do Sul, RS (EL), São Miguel das Missões, RS (SMM), and Guarapuava, PR (GP), with no grazing (NG) and grazing (G) of cover crops in integrated crop-livestock systems in subtropical Brazil. MDPA: internal standard Methylenediphosphonic acid. o: orthophosphate, p: pyrophosphate, m: myo-Inositol hexakisphosphate, g: D-glucose-6-phosphate, a: α-glycerophosphate, b: β-glycerophosphate, A: adenosine 5′ monophosphate, s: scyllo-Inositol hexakisphosphate, d: diester. Unidentified peaks between 5.7 ppm and 3.9 ppm were considered other monoesters. The spectra were vertically scaled (normalization scale) using as reference EL-G, which contained 0.0215 μg P as MDPA.

because the theoretical basis that oriented us was that greater biological complexity would enable better expression of ecosystem functions. Different soil characteristics, mainly derived from different texture and parent materials (i.e. basalt for Guarapuava and São Miguel das Missões and granite for Eldorado do Sul), led us to analyze individual samples from each trial for the ^{31}P NMR spectroscopy. However, to increase the strength of the statistical output of the P forms, functional groups and compounds, locations were combined as replicate blocks. Responses derived from ^{31}P NMR spectroscopy were consistent across locations, suggesting comparisons were valid across locations despite perceived differences.

Integrated crop-livestock systems promoted greater P output from production components, resulting in a lower P budget (i.e. more P taken off). This is in agreement with Costa et al. (2014), who also

Table 3
Soil phosphorus forms and functional groups estimated by ^{31}P nuclear magnetic resonance in NaOH-EDTA extracts of samples from Guarapuava, São Miguel das Missões, and Eldorado do Sul with no grazing (NG) (sole-cropping system) and grazing (G) of winter cover crops in an integrated crop-livestock system in subtropical Brazil.

Locate	Guarapuava		São Miguel das Missões		Eldorado do Sul		Mean across locations[d]	
Treatment	NG	G	NG	G	NG	G	NG	G
	mg P kg^{-1} soil							
Total P	2531	2372	1523	1530	841	1045	1632	1649
Inorganic P	2061	1953	963	1078	640	902	1221	1311
Organic P	470	419	561	452	201	143	411	338*
Residual[a]	1410	1542	278	210	201	185	630	646
Deg[b]	109	105	155	169	37	39	100	104
cMonoesters[c]	340	291	357	283	158	104	285	226**
cDiesters[c]	130	128	204	169	43	39	125	112
	%							
Inorganic P	81.4	82.8	63.2	70.5	76.1	86.3	73.6	79.9*
Organic P	18.6	17.7	36.8	29.5	23.9	13.7	26.4	20.3•
Residual[a]	36.4	40.2	14.1	12.3	20	15.6	23.5	22.7
Deg[b]	4.3	4.5	10.1	11	4.4	3.7	6.3	6.4
cMonoesters[c]	13.4	12.3	23.4	18.5	18.8	10	18.5	13.6•
cDiesters[c]	5.1	5.4	13.4	11	5.1	3.7	7.9	6.7
cMonoesters to cDiesters ratio	2.6	2.3	1.6	1.5	3.7	2.7	2.6	2.2

[a] Proportion of the P non-extracted with NaOH-EDTA.
[b] Deg: degradation peaks (di-esters converted to mono-esters), including α- and β-glycerophosphate.
[c] "c": corrected for degradation peaks. cMonoesters included all monoesters less α- and β-glycerophosphate. cDiesters included diesters plus α- and β-glycerophosphate.
[d] Significance codes between agricultural complexity levels: $^{**}p < 0.01$, $^{*}p < 0.05$ and $^{•}p < 0.1$.

Table 4
Soil phosphorus compounds estimated by ^{31}P nuclear magnetic resonance in NaOH-EDTA extracts of samples from Guarapuava, São Miguel das Missões, and Eldorado do Sul with no grazing (NG) (sole-cropping system) and grazing (G) of winter cover crops in an integrated crop-livestock system in subtropical Brazil.

Locate	Guarapuava		São Miguel das Missões		Eldorado do Sul		Mean across locations[b]	
Treatment	NG	G	NG	G	NG	G	NG	G
P compound	mg P kg^{-1} soil							
ortho	1968	1856	934	1038	637	902	1180	1265
pyro	93	97	29	41	3	ND	42	46
myo	181	137	149	96	102	77	144	103**
gluco	24	19	22	14	ND	4	15	12
α-glyce	63	60	89	94	21	25	58	60
β-glyce	46	45	66	75	16	14	43	45
Adeno	70	62	86	69	17	ND	58	44**
scyllo	64	60	69	60	38	24	57	48*
diester	21	23	49	ND	6	ND	25	8
other	ND	13	32	44	ND	ND	11	19•
	NaOH EDTA %[a]							
ortho	77.8	78.7	61.3	67.8	75.7	86.3	71.6	77.6•
pyro	3.7	4.1	1.9	2.7	0.4	ND	2	2.3
myo	7.2	5.8	9.8	6.3	12.2	7.3	9.7	6.5*
gluco	1	0.8	1.4	0.9	ND	0.4	0.8	0.7
α-glyce	2.5	2.6	5.8	6.1	2.5	2.4	3.6	3.7
β-glyce	1.8	1.9	4.3	4.9	1.9	1.3	2.7	2.7
Adeno	2.8	2.6	5.6	4.5	2.1	ND	3.5	2.4•
scyllo	2.5	2.5	4.5	3.9	4.6	2.3	3.9	2.9
diester	0.8	1	3.2	ND	0.7	ND	1.6	0.3
other	ND	0.5	2.1	2.9	ND	ND	0.7	1.1

ND: not detected, Ortho: Orthophosphate, Pyro: Pyrophosphate, myo: myo-Inositol hexakisphosphate, gluco: D-glucose-6-phosphate, α-glyce: α-glycerophosphate, β-glyce: β-glycerophosphate, Adeno: Adenosine 5′ monophosphate, scyllo: scyllo-Inositol hexakisphosphate, diester: Diester, other: Other monoesters between 5.7 ppm and 3.9 ppm excluding identified peaks.

[a] Percentage in relation to total P NaOH EDTA.

[b] Significance codes between agricultural complexity levels: $^{**}p < 0.01$, $^{*}p < 0.05$ and $^{•}p < 0.1$.

found lower P budget with ICLS compared to a sole-crop system. Even though output was greater in ICLS (grazed system), soil total P concentration in the 0–5 cm depth was not altered by agricultural complexity. This suggests that even with grazing having a greater P output, it was not enough to deplete soil P from the soil surface layer (0–5 cm) in comparison to the sole-cropping system. In contrast, Costa et al. (2014) found lower soil P stock with grazing than without grazing at soil depth of 0–20 cm in an oxisol located in subtropical Brazil.

It is important to state that although we found lower C concentration in ICLS soils (0–5 cm) than soil under a no-tillage ungrazed cover crop system, this result does not imply a depletion of soil C stocks in subtropical Brazil. Evaluation at greater soil depth would be needed to accurately characterize soil organic C stock change. In the same region on a Ferrasol, Piva et al. (2014) found that soil organic C stock was not different between sole-cropping and ICLS at 0–20 cm or at 0–100 cm. Martins et al. (2014a) also found no difference in soil organic C at a depth of 0–40 cm between sole-cropping and moderate grazing of an ICLS in an oxisol located in subtropical Brazil. Soil organic C stock could become depleted if grazing intensity were too high.

Many soil chemical characteristics were not different between grazing and no grazing treatments across locations. However, soil P compounds estimated by ^{31}P nuclear magnetic resonance gave us clues as to how to better understand agricultural complexity effects on soil P composition (Tables 3–4). Grazing resulted in lower organic P and greater inorganic P concentrations, including its bioavailable fraction (Mehlich-I P), than without grazing. Moreover, in the organic form, lower inositol phosphates (myo- and scyllo-Inositol hexakisphosphate) concentration occurred with grazing. However, the molybdate-reactive P was greater with grazing only in Eldorado do Sul. For the other locations, as well as across locations, molybdate-reactive P and molybdate-unreactive P pools did not differ between agricultural complexity treatments. Our hypothesis that livestock grazing would increase P availability in soil was supported. Therefore, land-use intensification with ICLS may be a strategy to enhance P use efficiency in agro-ecosystems.

Phosphorus has chemical affinity properties that make it relatively unavailable in many soils. In particular, myo-Inositol hexakisphosphate concentration has very low bioavailability to plants. In acidic weathered soils (e.g., oxisols), P is largely sorbed to Fe and Al oxides (McDowell, 2012). In addition, as described in Nash et al. (2014), inositol phosphates have greater affinity than orthophosphate for Fe oxides (Martin et al., 2004). In our study, agricultural complexity had a minor effect on Al_{ox} and Fe_{ox}, differing only at Eldorado do Sul, where concentrations were greater with grazing. Possibly soil inositol phosphate concentrations were more influenced by inputs through the soil-plant-animal cycling than by soil Al_{ox} and Fe_{ox} concentrations. However, soil oxyhydroxide concentrations may have had an important role on fixing soil inositol phosphates added to the soil, making them weakly available to microorganisms. High anionic charge of inositol phosphates, particularly myo-Inositol hexakisphosphate, facilitates the formation of strong electrostatic bonds (Menezes-Blackburn et al., 2012; Turner et al., 2002). Bonds between myo-Inositol hexakisphosphate and soil constituents like goethite (iron oxyhydroxide) have features of both inner and outer sphere complexes, with the balance dependent on soil pH (Nash et al., 2014).

A contributing factor to myo-Inositol hexakisphosphate accumulation is that ungrazed cover crops can produce a greater quantity of seed than those that are continuously grazed. Phytic acid is synthesized by plants and is stored primarily in seeds where it can represent 60 to 80% of total plant P (Nash et al., 2014), so myo-Inositol hexakisphosphate enters the soil through direct deposition of plant material (Noack et al., 2012). Turner and Blackwell (2013) also reported that ungerminated seeds might provide an additional input of myo-Inositol hexakisphosphate to soils. This is in contrast to grazed cover crops, because a greater part of biomass is returned as cattle and sheep dung; which have the majority of P content (>80%) as orthophosphate (McDowell et al., 2006). Sheep and cattle are able to hydrolyze phytate (Humer and Zebeli, 2015), and this is one of the reasons why manure has high orthophosphate levels, which may also be readily available to plants and microorganisms relative to phytate.

Greater orthophosphate accumulation in soil favors dissolved P losses via surface or subsurface leaching flow. However, this phenomenon was not expected to be significant in our experiments, since soils had high Al_{ox} and Fe_{ox} contents and were not close to P saturation. Our calculations indicate P saturation was below critical levels for significant mobilization of P in runoff. The main factor related to dissolved P release or loss is soil P solubility, and this depends on P quantity added to soil and soil's ability to retain P (McDowell, 2012).

Since nutrients in cover crop forages were ingested by animals, passed through their digestive tract, and deposited as feces (and urine) on soil, rapid transformation of organic P forms to orthophosphate can lead to immediate nourishment of subsequent crops. This may be dependent on feces deposition pattern on the pasture and feces decomposition rate, as influenced by temperature and rainfall. In addition, low animal-product export of P means that up to 85% of ingested P through pasture consumption is returned via dung (Nash et al., 2014). As described above, most cattle and sheep dung P occurs as inorganic P. According to our results, livestock grazing of cover crops increased soil total inorganic P concentration, total orthophosphate concentration and bioavailable orthophosphate concentration (Mehlich I).

In addition to the effect of grazing livestock on P cycling through dung deposition, livestock can impact plant dynamics and diversity of soil biological functioning, which can feed-back on the plant community and eventually on animal production. Compared with no or excessive grazing, moderate grazing can stimulate tillers and root production by shoot renewal (Moraes et al., 2014), the effect of which can increase soil organic C stock (Salton et al., 2005). Moreover, grazing promotes intensive exudation of organic compounds by roots (Tisdall and Oades, 1982; Hamilton et al., 2008), which are energy sources for soil microorganisms (Moraes et al., 2014). Other investigations have demonstrated that ICLS compared with simpler cropping practices promoted greater microbial biomass, phosphatase activity (Acosta-Martínez et al., 2010a, 2010b), and microbial functional diversity (Chávez et al., 2011). Greater microbial biomass and diversity could have enabled hydrolysis of more soil organic P compounds in ICLS through enzymatic processes, but further tests are needed to verify this occurrence in Subtropical Brazil.

In addition to producing similar or greater grain yield with grazing of cover crops and similar fertilization doses (Moraes et al., 2014), ICLS produced meat and other animal-derived products, such as milk and wool, obtaining greater animal productivity without supplemental feed on pasture (only mineral salt) (Carvalho et al., 2010; Moraes et al., 2014; IBGE, 2015). Therefore, we can state that efficient use of nutrients was achieved in ICLS with greater overall output.

5. Conclusions

Enhanced agricultural complexity promoted by grazing in ICLS increased bioavailable orthophosphate concentrations and reduced recalcitrant organic P as myo- and scyllo-Inositol hexakisphosphate. Therefore, integrating livestock with annual crops can be a sustainable alternative to improve soil P availability, even with greater P output in farming systems of subtropical Brazil.

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CHAPTER 2

Soil organic phosphorus dynamics on terrestrial natural ecosystems: a meta-regression analysis

Soil organic phosphorus dynamics on terrestrial natural ecosystems: a meta-regression analysis

Abstract

Soil organic phosphorus (P) and its functional groups can be modified by distinctive ecosystems properties. This study aimed to analyze soil organic P dynamics on natural ecosystems through meta-regression, among different soil textures and locates at global scale, relating its organic functional groups with other important soil characteristics. A search was made up to identify published papers that have determined soil P composition by liquid state one-dimensional ^{31}P nuclear magnetic resonance of soils extracted with NaOH EDTA. An eligibility criteria was used to select papers and moreover, results were categorized into groups according to pre-defined factors locate (i. between and ii. below-above Capricorn and Cancer Tropics) and soil texture (non-clayey soils and clayey soils). This analysis enabled a comprehensive understanding of soil P dynamics at an unprecedented geographical scale. Our results were that total soil organic P concentration had no association with soil pH, carbon (C) concentration, carbon-to-nitrogen (CN) ratio and carbon-to-phosphorus (CP) ratio. Monoesters-to-organic P ratio increased exponentially as pH increased and decreased linearly as C concentration, CN ratio, and CP ratio increased. In contrast, diesters-to-organic P ratio as well as diesters-to-monoesters ratio had the opposite behavior, decreasing exponentially as pH increased and increasing linearly as C concentration, CN ratio, and CP ratio increased. Diesters-to-organic P ratio versus soil C concentration was the only response that interaction among locates and soil textures occurred. As soil C concentration increased, diesters-to-organic P increased in a higher degree on clayey soils when compared to non-clayeyed ones on locates below-above Capricorn and Cancer Tropics, and no effect was observed for locates between Capricorn and Cancer Tropics. Therefore, soil pH, C concentration, CN ratio and CP ratio are important factors in determining soil organic P functional groups composition on natural ecosystems. Finally, as soil organic P functional groups showed contrasting behavior in response to those soil variables, we conclude that it needs to be studied by its functional forms in order to understand its dynamic, instead of a unique entity.

1. Introduction

Natural ecosystems functioning rely on phosphorus (P) cycling. All living beings need, in higher or lower degree, P availability to its nutrition. Land-based ecosystems are mainly influenced by P composition in soils, which is a good indicator of how ecosystem is functioning. In soils, P is distributed into organic and inorganic forms and these are subdivided into fractions or functional groups. Phosphorus bioavailability for fauna and flora depends on which species (into the functional groups) the nutrient is present in soil as well as phosphatase enzymes presence to break down soil organic P molecules. Plants and microorganisms can uptake phosphorus from soil solution when it is present as free orthophosphate. The main functional groups of P species founded in soils constituents of organic form are orthophosphate monoester, orthophosphate diester, phosphonate and orthophosphate anidre (organic polyphosphate) (Condron et al., 2005) and of inorganic form are orthophosphate, polyphosphate and pyrophosphate (Cade-Menun and Preston, 1996). To be able to uptake organic P from soil, plants and microoganisms need to breakdown organic P until orthophosphate is released in soil solution. Diesters require hydrolysis by both phosphodiesterase and phosphomonoesterase, whereas monoesters require only the last one (Turner, 2008b).

The processes determining P cycling in soils are complex. The main factors controlling it are the quality of the plant residues, the activity of the soil microbial biomass (Damon et al., 2014), reactions of P in soil and soil pH. The P release from plant residues and subsequent availability to plants depends on the following processes: I) the rate of release of the inorganic and organic P fractions from both residues and the microbial biomass pool, II) the assimilation of bioavailable orthophosphate by the microbial biomass as it proliferates after the addition of plant residues, and III) the uptake of native soil orthophosphate by the stimulated microbial biomass when orthophosphate released from plant residues is lower than microbial community requirement after litter addiction (II) (Damon et al., 2014).

Soil properties have mandatory control over soil P composition. These properties are likely to be similar to those controlling the accumulation and stabilization of soil organic matter. Organic and inorganic P in soils can be attached to clays and Al and Fe oxyhydroxides, which depend on sorption and desorption processes to release

orthophosphate to soil solution. Moreover, inorganic P can also be present as secondary (e.g., Ca, Fe and Al phosphates) and primary P minerals (e.g., apatites), depending on dissolution processes to release orthophosphate, and precipitation as a way to fix P in the case of secondary minerals (Shen et al., 2011). Total soil organic P concentration tended to increase along with a relative enrichment of P in the organic matter as particle size decreased (Rubæk et al., 1999; Makarov et al., 2004). Finally, investigations have shown that acidic soils have greater proportion of the organic P as diesters, and neutral soils have lower proportion of monoesters in both tropical and temperate conditions (Turner and Engelbrecht, 2011; Turner and Blackwell, 2013).

The potential influence of locates with different climates and particular soils textures on soil organic P dynamics is not a simple question to address. Beyond basic characteristics such as temperature, rainfall, soil texture and clay texture, other factors like site-specific fauna and flora can modify P dynamics (e.g., Turner et al., 2014). As described in Turner et al. (2002) and references therein, soil organic P concentration decreases with increasing temperature, because higher temperatures are optimal for the breakdown of soil organic P by the microbial biomass and phosphatase enzymes. The extent of soil drying can also exert a considerable control over inositol phosphates solubility, which is a recalcitrant organic P compound, due to physical disruption mechanisms involving soil structural changes (Turner et al. 2002). Another factor that could contribute to soil organic P dynamic is the stage of pedogenesis. Ecosystems development stage has an influence over the soil P composition, since soil changes occur as soil ages. These changes include soil Al and Fe oxides concentrations (Turner et al., 2014; Turner et al., 2007), soil C:N:P:S stoichiometry (Walker, 1965, Turner and Condron, 2013), and changes in inputs to soil, either from plant or microbial communities (Jangid et al. 2013; Turner et al. 2012; Turner et al., 2014). Soil P limitation may increase phosphoesterases synthesis as a way to increase organic P breakdown to bioavailable P (Turner and Haygarth, 2005; Kunito et al., 2012).

Soil organic P composition has been studied in soils from ecosystems around the world and nuclear magnetic resonance spectroscopy (NMR) was a widely used method to access soil organic P species (Kizewski et al., 2011). This technique can be used for both qualitative and quantitative estimates of P species in soil (Cade-Menun and

Preston, 1996) and the more effective extractant of P species for NMR analysis has been NaOH plus the chelating agent EDTA (Cade-Menun and Liu, 2013). This do not implies that NaOH EDTA is the best extractant for ^{31}P NMR, however, by its wideness use, it is a good baseline for comparison (Cade-Menun and Liu, 2013). According to Cade-Menun and Preston (1996) the NaOH can solubilize organic and complex inorganic P while EDTA chelates metallic cations to increase P extraction efficiency from soil. The NaOH EDTA extraction method is recognized to quantitatively extract organic P from soil, because its extract concentrations are similar to other methods considered to provide accurate measurement of soil organic P (Turner and Blackwell, 2013). However, the method onerousness and complexity make it difficult for scientists to access large number samples in their research.

There is a lack of a broader understanding of how soil organic P composition is affected by these soil variables because responses that are already known were obtained from case-specific conditions. Knowing how soil characteristics affect soil P composition at a larger geographical scale could enable a comprehensive understanding soil organic P dynamics. Therefore, we proposed to combine soil organic P results obtained from ^{31}P NMR using NaOH EDTA, to be able to understand how soil organic P composition is determined in natural ecosystems. Our hypothesis was that soil organic P and its functional groups can be modified by distinctive ecosystems properties, in different locates and soil textures across the world. The objective was to analyze through meta-regression, soil organic P dynamics on natural ecosystems, among different soil textures and locates at global scale, relating its organic functional groups with soil pH, carbon (C) concentration, carbon-to-nitrogen (CN) ratio and carbon-to-phosphorus (CP) ratio.

2. Methods

A search was made up to September 15, 2014, to identify published papers that accurately determined soil P species by liquid state one-dimensional ^{31}P NMR on NaOH EDTA extracts. According to McDowell et al. (2006) and Cade-Menun and Liu (2013), we are considering here as accurate papers, those ones that estimated an adequate delay time prior to NMR analysis, enabling therefore, to produce quantitative data on the NMR

instrument. We used two platforms and specific search terms for each one. The first platform was the Web of Knowledge, where it was used the following terms: “soil* phosphorus or P or 31P* nuclear-magnetic-resonance or NMR* naoh or sodium hydroxide* edta or ethylenediaminetetraacetic” from which was obtained 107 results. The second platform was the Google Scholar, where it was used the following terms: “soil* phosphorus* “nuclear magnetic resonance”* naoh* edta” resulting in 1510 results (non including patents and citations).

We followed a pre-defined eligibility criteria to consider the papers, and after to select or reject those considered papers. To consider the papers, we started evaluating the title, passing to the abstract when the first it was not enough and the whole paper when the two previous did not clarify our requirements. The eligibility criteria used to select or reject papers had the following steps: I) original field soil samples (manure, pot soil, soil leachate and sediment samples were excluded) and when studies changed natural conditions, only control (unchanged) was used, i.e., original condition (e.g., litter removal in Vicent et al., 2010 was excluded); II) Samples which were analyzed by liquid state one-dimensional ^{31}P NMR, according to the following features: a) NaOH EDTA extractor without pretreatment (0.5 or 0.25 M NaOH and 0.1 or 0.05 M EDTA), b) delay times > 2.00 s (i.e., quantitative data, see Cade-Menun and Liu, 2013). Papers that did not show c) NMR features or explanation according to ^{31}P NMR principles (see Cade-Menun and Liu, 2013) to use delay times lower than 2.0 s were excluded. Papers that did not show d) total NaOH EDTA extracted P and total P were also excluded.

Moreover, results from native vegetation soils (native forest or scrub) were categorized into groups according to pre-defined factor levels. These factors were geography and soil texture. Geography was used because it is know that soil organic P concentrations decrease with increasing temperature, because higher temperatures are optimal for the breakdown of soil organic P by the microbial biomass and phosphatase enzymes (Turner et al., 2002). Into geography factor, results were categorized in two levels, as I) between Capricorn and Cancer Tropics and II) below-above Capricorn and Cancer Tropics (locate will be used to identify this factor afterwards). By doing this separation we expected to have more influence on those locates of higher and lower temperatures, respectively. Soil texture was also used as a categorical factor because it

is know that clay concentration has a major influence controlling soil organic P dynamics in soils (Rubæk et al., 1999; Makarov et al., 2004; Nash et al., 2014). For soil texture, we used a criteria adapted from McSherry and Ritchie (2013), where the results were categorized as non-clayey soils (<35% clay) and clayey soils (>35% clay). It was not possible to analyze clay concentration as a continuous factor because some papers just categorized clay into clay classes (e.g., sandy loam, sandy, clayey). When the paper did not present information that allowed us to make this categorization, it was excluded. Only surface sample results were used when more than one depth existed.

The results extracted from selected papers were: NaOH EDTA total extracted P, NaOH EDTA organic P, monoesters-to-organic P ratio, diesters-to-organic P ratio as well as other basic soil characteristics: total P, total C, total nitrogen (N) and pH. To extract these results we used the following conversions and assumption: and b) $CaCl_2$ pH was converted to H_2O pH adding 0.7 pH units; and from KCl pH was used a factor of 1.15 to convert to H_2O pH, e) when total C was unavailable, organic C was assumed as total C. It was not found any duplicity on the papers selected, i.e., repeated results in different papers. Not all results had soil C and N concentrations and the plots number used for each analysis is presented on the results section. We know that it is possible to correct degraded peaks of diesters converted to monoesters (e.g., Young et al., 2013 and Cade-Menun et al., 2010), but since some papers only showed fractions like monoesters and diesters, and not species inside these functional groups, this correction wan not done. Doolette et al. (2009) showed that monoesters α- and β-glycerophosphate are diesters derived species, products of phospholipid hydrolysis occurred during extraction process.

Meta-regression analysis was conducted on R Version 3.1.0 (© 2014 The R Foundation for Statistical Computing). Statistical models were adjusted considering variables as outcome measures in decimal units, where 1 = 100 % (Yasiry and Shorvon, 2014). It was used a factorial design including as moderators the categorial predictors soil texture and locate and, individually per analysis, the continuous predictors soil pH, C concentration, CN ratio and CP ratio. Those were fitted to the random-effects model with the restricted maximum-likelihood estimator, using metafor-package (Viechtbauer, 2010). Based on Wald-type chi-square test, we verified factors and interactions effects significances. Where any factors or interactions effect were detected, we excluded

factors and interaction for the model and used only the covariate as model moderator, to test covariate effect overall results. A likelihood ratio test (LRT) was used to test the significance of excluding moderators.

Variables heterogeneity and publication bias were verified. Heterogeneity was assessed according to (i) the estimated amount of residual heterogeneity, i.e., τ^2 (tau-squared), (ii) the I^2 statistic, which estimates how much of the total variability in the effect size estimates can be attributed to heterogeneity among the true effects, and (iii) the significance of the test for residual heterogeneity (*Q*M) (Viechtbauer, 2010). Presence of publication bias was evaluated by contour-enhanced funnel plots (standard error, variances and its respective inverse values) (Peters et al., 2008) and results were excluded when exceeded p-values below 0.01.

As almost all the results are from single nuclear magnetic resonance spectra, they did not have standard errors or variances. So we used fixed variances for each variable determined among all results. This is a practice similar to the ones used in other studies where standard errors or variances were not available (Parmesan and Yohe, 2003; Georges and Fossette, 2006). However, this approach is suboptimal, because it ignores the different amounts of information that studies of different sizes and qualities present (Stewart, 2010). Then, we did use as weight, the percentage of P extracted with NaOH EDTA, using the inverse of the variance method. Weighting the data according to their levels of precision is recommended, as suggested by Hedges and Olkin (1985) (Philibert et al., 2012). The NaOH EDTA extraction process should attain the maximum non-reactive P extraction (Cade-Menun and Liu, 2013). We assumed that the higher the NaOH EDTA extraction was, better experimental conditions were used on those experiments, to achieve the maximum non-reactive P extraction (i.e., pretreatment, soil to solution ratio and time of extraction).

3. Results

Our search resulted in 81 native vegetation top-soil results from nine references (Backnäs et al., 2012, n=1; Celi et al., 2013, n=2; McDowell and Steward, 2006, n=4; McDowell et al., 2007, n=26, Turner and Engelbrecht, 2011, n=19; Turner et al., 2003, n=1, Turner et al., 2007, n=8; Turner et al., 2014, n=10; Turner, 2008, n=1; Vicent et al.,

2010, n=1; Vicent et al., 2013, n=8). From which 46% referred to locates between Capricorn and Cancer Tropics, being 11% non-clayey soils (<35% clay) and 89% clayey soils (>35% clay). And the remaining 54% were of locates below-above Capricorn and Cancer Tropics, being 39% non-clayey soils and 61% clayey soils. The reasons why most of the papers were excluded (from the ~1500 papers found) were that papers didn't fulfill aspects of the eligibility criteria including, for example, other land uses (e.g., crop, pasture, planted forest, or wetlands), and NMR features.

Regarding publication bias, only three results were excluded from analysis, because they exceeded p-values below 0.01, and referred to non-clayey soils from locates below-above Capricorn and Cancer Tropics. For all variables, considering models including factors and its interaction as well as interaction subsets models, heterogeneity results were τ^2 <0.001, I^2 < 0.01% and QM ($p \geq 0.99$).

There was no effect of pH ($p = 0.22$) on total organic P concentration (LRT, $p = 0.11$). However, pH affected, overall results (for all three LRT, p raction smonoester to organic P ratio, diesters-to-organic P ratio and diesters-to-monoesters ratio (for all three, $p < 0.001$). Predicted monoesters-to-organic P ratio increased exponentially as pH increased ($y = -0.037\ e^{0.52\ pH}$). Whereas predicted diesters-to-organic P ratio ($y = 0.95\ e^{-048\ pH}$) and diesters-to-monoesters ratio ($y = 1.73\ e^{-0.92\ pH}$) had the opposite behavior decreasing exponentially as pH increased (Figure 1).

Soil C concentration ($p = 0.22$) had no effect on soil organic P (LRT, $p = 0.25$). Monoesters-to-organic P ratio was influenced overall results ($p < 0.0001$) (LRT, $p = 0.29$) by soil C concentration, and its predicted concentration decreased 0.63% (se ± 0.12%, intercept = 88.4%) for each unit soil C concentration increased. For diesters-to-organic P ratio, there was a significant interaction among locates and soil textures ($p = 0.1$). The main difference among treatments was the no effect of soil C concentration, for both soil textures, on locates between Capricorn and Cancer Tropics (for two soil textures $p > 0.32$). Whereas on locates below-above Capricorn and Cancer Tropics, predicted diesters-to-organic P ratio increased as soil C concentration also increased, in a higher degree for clayey soils. The increase on non-clayey soils was 0.65% (se ± 0.14%, intercept = 7.8%) and on clayey soils was 0.71% (se ± 0.15%, intercept= 8.7%) for each unit soil C concentration increased. Predicted diesters-to-monoesters ratio increased

overall results 0.013 units (se ± 0.002) for each unit soil C concentration increased ($p < 0.0001$) (LRT, $p = 0.99$) (Figure 2).

Analysis of CN and CP ratios was made with a smaller dataset, because not all papers provided soil N and C concentrations. Fortunately these numbers of abstentions were low. Analysis using as covariate CN ratio was conducted with n = 72 and CP ratio with n = 77, both considering the exclusion of three results considered as publication bias (described earlier). CN ratio was analyzed with 19% non-clayey soils and 81% clayey soils from locate between Capricorn and Cancer Tropics (29%). And locate below-above Capricorn and Cancer Tropics (71%) had 53% non-clayey soils and 47% clayey soils. CP ratio from locates between Capricorn and Cancer Tropics (27%) had the same data proportion described for CN ratio. And locates below-above Capricorn and Cancer Tropics (73%) had 52% non-clayey soils and 48% clayey soils.

It was not detected effect of CN ratio on organic P concentration ($p = 0.48$) (LRT, $p = 0.12$). CN ratio had an effect ($p< 0.0001$) overall results (LRT, $p =0.34$) on monoester to organic P ratio, in which predicted results decreased 0.9% (se ± 0.2%) for each CN ratio unit increased. the locate effect was significant for both diesters-to-organic P ratio and diesters-to-monoesters ratio. For both variables, locates between Capricorn and Cancer Tropics had no CN ratio influence. However, for below-above Capricorn and Cancer Tropics, as CN ratio increased, diesters-to-organic P ratio increased 1.07% (se ± 0.2%, intercept=-2.9%) and diesters-to-monoesters ratio increased 0.02 units (se ± 0.004, intercept=-0.16) (Figure 3).

Finally, CP ratio had no effect on organic P ($p = 0.26$) (LRT, $p = 0.26$). Effect was detected overall results (for all following three, $p < 0.0001$) for monoester to organic P ratio (LRT, $p = 0.23$), diesters-to-organic P ratio (LRT, $p = 0.26$), and diesters-to-monoesters ratio (LRT, $p = 0.26$). Monoesters-to-organic P ratio decreased 0.04% (se ± 0.01%, intercept=87.7%), and both diesters-to-organic P ratio (0.04%, se ± 0.01%, intercept=8.3%) and diesters-to-monoesters ratio (0.0008, se ± 0.0001%, intercept=0.06) increased as CP ratio increased (Figure 4).

4. Discussion

Meta-regression analysis may be used to understand ecological relations, in a broad sense, where studies were developed in case-specific contexts with a reduced number of plots. This can be mandatory for research areas where results are not simple to acquire and instruments or methods are not broadly accessible for scientists, such as the ^{31}P NMR. Moreover, where single studies were not enough to generate a wide concept or different studies present contradictory results of similar phenomena.

In our meta-regression, the soil organic P concentration had no influence of soil pH, C concentration, CN ratio and CP ratio; however, soil organic P composition had a complex response to those soil characteristics. Monoesters-to-organic P ratio increased as pH increased, and decreased as C concentration, CN ratio and CP ratio increased, with no particular response among locates and soil textures. In contrast, diesters-to-organic P ratio as well as diesters-to-monoesters ratio had the opposite behavior, decreasing its concentrations as pH increased, and increasing as soil C concentration, CN ratio and CP ratio increased. However, with exceptions for the non-effect on locates between Capricorn and Cancer Tropics for both (i) soil C concentration on diesters-to-organic P ratio and (ii) CN ratio on diesters-to-organic P ratio and diesters-to-monoesters ratio. Finally, a particular soil texture response of diesters-to-organic P as a function of soil C concentration on locates below-above Capricorn and Cancer Tropics occurred. As soil C concentration increased, diesters-to-organic P increased in a higher degree on clayey soils when compared to non-clayeyed ones. Thus, the meta-analytic method used enabled us to distinguish particular responses of soil organic P functional groups as a function of other soil characteristics, among locates and soil textures, taking into account basic requirements of meta-analysis, such as results homogeneity, and publication bias exclusion (Figures 1, 2, 3 and 4).

For instance, this is the first paper that proposes a clear relationship of soil organic P compounds and soil pH, soil C concentration, CN ratio, and CP ratio, at a large geographical scale including a diversity of natural ecosystems. Our findings of pH influence on soil organic P functional groups corroborate with Turner and Engelbrecht (2011) for tropical forest soils, where the most acidic soils contained greater proportion of the organic P as diesters (mainly DNA), while neutral soils contained greater proportion as monoesters (with negligible inositol hexakisphosphate contribution). It also supported

Turner and Blackwell (2013) results for temperate arable soils, where greater proportion of DNA in acidic soils and greater proportion of monoesters (non-inositol hexakisphosphate) in neutral soils were found. Moreover, as demonstrated for temperate pasture soils, phosphomonoesterase activity was higher in acid soils and phosphodiesterase was higher as pH increased (Turner and Haygarth, 2005). Therefore, this could have contributed to break down organic P molecules where its required enzymatic activity was greater.

In response to soil characteristics, specific soil organic P species (e.g., inositol phosphate), rather than its functional group response (i.e., inositol is a specie of monoesters functional group), could have contributed to a greater dispersion of results on the meta-analytic scatter plot. As it was described earlier, it was not possible to analyze the organic P species, since not all papers showed all species concentrations. For example, although inositol phosphates are classified as monoesters, they could have had a different response when compared to other monoesters (or monoesters-to-organic P ratio), because its molecular structure makes unique the consequent dynamic on the environment. Turner and Blackwell (2013) investigating results from temperate arable-soils verified that inositol phosphate concentrations were greater in the most acidic soils, what they attributed to a greater potential for stabilization in this condition. This goes in divergence to our results, because we presented that total monoesters as fraction of organic concentration, obviously including inositol phosphates if it was present, decreased its concentration as soil acidity increased. Consequently, specific responses of P species could have contributed to greater dispersion on organic P functional groups as a function of soil pH.

Interaction among soil textures and locates was only detected for diesters-to-organic P ratio as a function of soil C concentration (Figure 2). There was no soil C effect on that functional group for both soil textures in locates between Capricorn and Cancer Tropics. One possible aggravating fact for the non-effect was the lower range of soil C concentration on this locate for both soil textures. This was expected because these plots were from low-land tropical forests, where usually relatively low soil C concentration occurs (Dieleman et al., 2013). However, in high-land tropical forest soils, where higher

soil C concentration is expected (Dieleman et al., 2013), still remain to be better know how soil organic P is composed by its functional groups.

Clayey soils had a greater rate of increase for diesters-to-organic P ratio than the non-clayey ones as soil C concentration increased on locates below-above Capricorn and Cancer Tropics (Figure 2). This could be related to the fact that diesters such as DNA can be protected from dephosphorylation by adsorption into the soil matrix (Nash et al., 2014), which may increase in clayey soils because of the higher surface area, and possibly higher iron and aluminium oxides. The amino groups protonation of adenine, guanine and cytosine bases in DNA molecule can cause adsorption of charged DNA by the charged clay surface (Yu et al., 2013).

As soil N and P limitation increased on land-based natural ecosystems, measured here by their ratios to soil C concentration, the monoester-to-organic P ratio decreased, and the diesters-to-organic P ratio as well as the diesters-to-monoesters ratio increased (Figures 3 and 4). The mechanisms that promoted the inverse response of monoesters and diesters as N and P limitation increased are possibly related to the development stage of those ecosystems. As time passes since onset of pedogenesis, the soil profile weight as organic C (100 times), N (10 times), sulfur (1 time) and P (1 time) tend to increase until achieve a maximum accumulation and then decrease according to the following order: P > N and sulfur > C; organic C has a lower decrease as ecosystems develop because its primary productivity continues to increase initially and decomposition is slowed by the increasingly acidic pH (Walker, 1965; Turner and Condron, 2013). Consequently, older systems may have higher CN and CP ratios, since the decaying degree of the first element is lower than the other ones, which may inhibit soil microbial activity and consequent decomposition, and because P and N are removed from the system following lixiviation.

Investigations have shown that diesters proportion, including phospholipids and DNA, increased as soil aged and monoesters concentration increased at beginning stages of pedogenesis and declined at older stages (Turner et al., 2014, Vincent et al., 2013, Turner et al., 2007, McDowell et al., 2007). To plants and microoganisms breakdown diesters, they need a higher investment for P acquisition than for monoesters, since it requires hydrolysis by both phosphodiesterase and

phosphomonoesterase to release free phosphate, whereas monoesters require only the last one, i.e., lower investment (Turner, 2008b). Moreover, according to Turner and Haygarth (2005) and Kunito et al. (2012), P limitation increased phosphoesterases synthesis as a way to increase organic P breakdown to bioavailable P. Therefore, as P turned to be more scarce, plants and soil microorganisms could have been stimulated to produce phosphomonoesterases in greater amount when compared to phosphodiesterase, because of the lower investment requirement for organic P acquisition. This could have led to monoesters depletion and increased diester proportion in soils from nutrient limited ecosystems.

Although inositol phosphates were also included on monoesters-to-organic P ratio, they need a higher investment in organic P acquisition than other monoesters and diesters, because they are strongly bounded to metal oxides, clays as well as organic matter, requiring both solubilization and hydrolysis by phytase to release a free orthophosphate (Turner, 2008b). However, according to Turner et al. (2014) and Turner et al. (2007), inositol phosphates declined to low concentrations in older soils, which in fine textured soils it was mirrored by a decline in amorphous Al and Fe oxides, what may have declined sorption sites for inositol phosphates and weakened protection from plant and microbial enzymatic attack. In contrast, coarse textured soils had an increase in Al and Fe oxides concentrations as soils aged, and inositol phosphates decline was attributed to changes in its inputs into soil, either from plant seeds or microbial synthesis, including through shifts in plant or microbial communities (Jangid et al. 2013, Turner et al. 2012 and Turner et al., 2014). Therefore, both soil and plant characteristics could have contributed to the inositol phosphates decline (here represented along with other monoesters) in older ecosystems depleted with soil P and N.

Based on our research synthesis and meta-regression analysis we observed that more native vegetation results are required from locates between Capricorn and Cancer Tropics, on a wider soil C concentration range, for both clayey and non-clayey soils, what can be achieved analyzing tropical high-lands soils, to enable a better understanding of P dynamics. Also more results are required on alkaline soils from both locates (between and below-above Capricorn and Cancer Tropics).

We expect for future researches to provide results of all soil organic P species results rather than functional groups only (e.g., as supplementary tables), even when species concentrations are low (and describe when species were not detected), what may enable future meta-analysis to avoid confounding effects of organic P species inside functional groups. And researchers may determine variances or standard errors for soils with distinctive properties. Then, as stated by Stewart (2010), future meta-analysis can use the different amounts of information that studies of different sizes and different quality present.

5. Conclusion

We conclude that soil pH, C concentration, CN ratio and CP ratio are important factors in determining proportions of soil organic P functional groups; however, refuting our initial hypothesis, these factors do not change total soil organic P concentration at global scale. Therefore, knowing how soil organic P composition is affected by those soil variables enabled a comprehensive understanding of soil P dynamics at an unprecedented geographical scale. Soil organic P dynamics cannot be clearly understood without considering diester and monoester functional groups.

As soil C concentration increase, diesters-to-organic P ratio increase in a greater rate in clayey soils when compared to non-clayey ones, on locates below-above Capricorn and Cancer Tropics, possibly because of the greater reactive soil surface availability to retain these compounds. Moreover, diesters proportion in soils tend to increase as ecosystems ages and get depleted of soil nutrients, due to a lower required investment by plants and microorganisms, to break down monoesters through their enzymes, to acquire soil organic P.

On locates between Capricorn and Cancer Tropics, C concentration have no influence on diesters-to-organic P ratio. On the same locates, diesters-to-organic P ratio and diesters-to-monoesters ratio do not change as CN ratio increase. These non-responses possibly occur because those soils are from low-land tropical ecosystems and they are at older stages of pedogenesis, where usually relatively low soil C concentration occurs. At this stage, possibly soil carbon concentration and soil N limitation is not controlling diesters transformations in soils.

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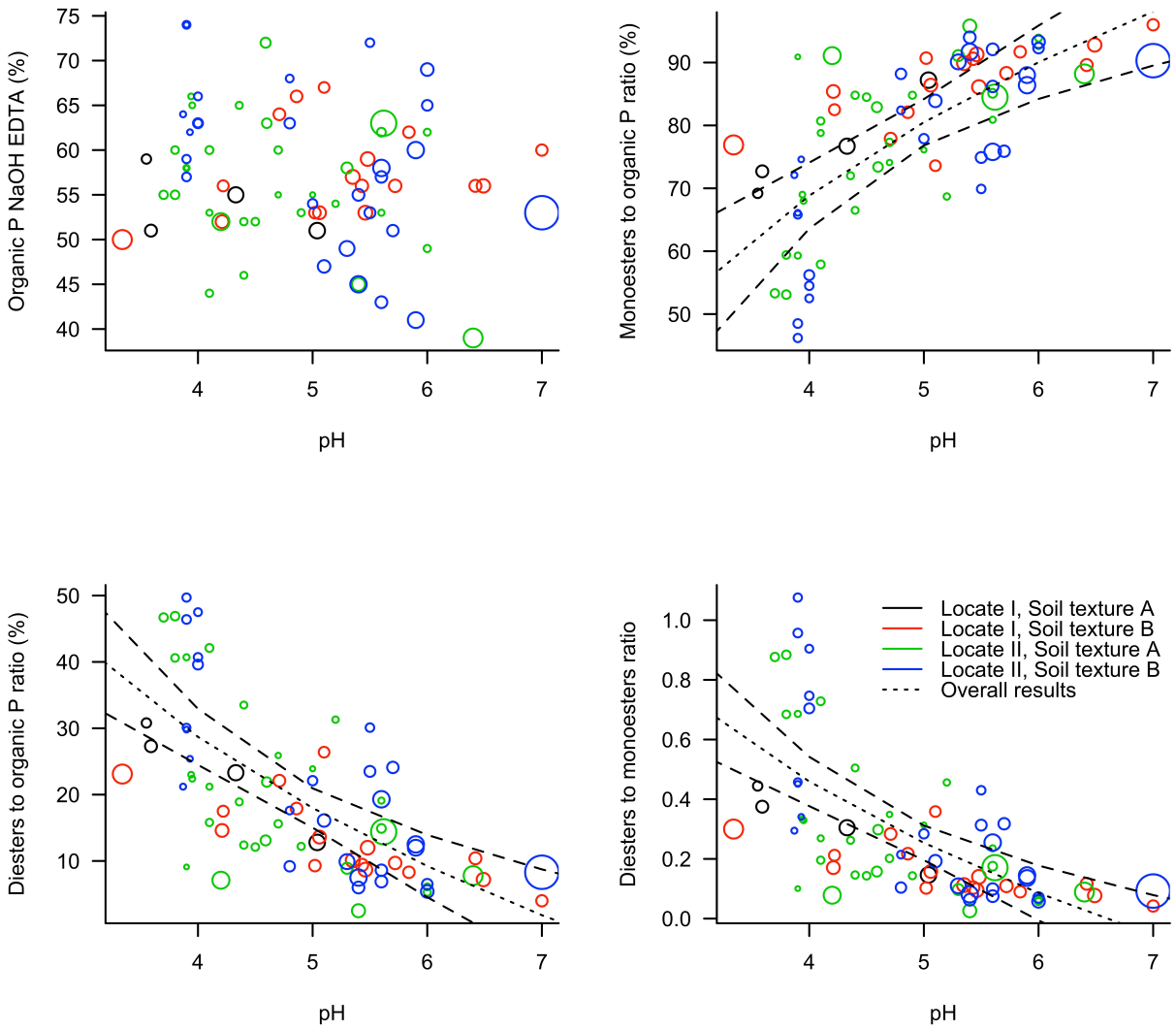

Figure 1. Soil pH influence on soil organic phosphorus dynamics on natural ecosystems. Locates were from (I) between and (II) below-above Capricorn and Cancer Tropics, while soil textures were (A) non-clayey soils (<35% clay) and (B) clayey soils (>35% clay). The point sizes were drawn proportional to the inverse of the weights (i.e., higher NaOH EDTA extraction are shown as larger points). Based on a mixed-effects model, the predicted responses with corresponding 95% confidence interval bounds were added to the plots.

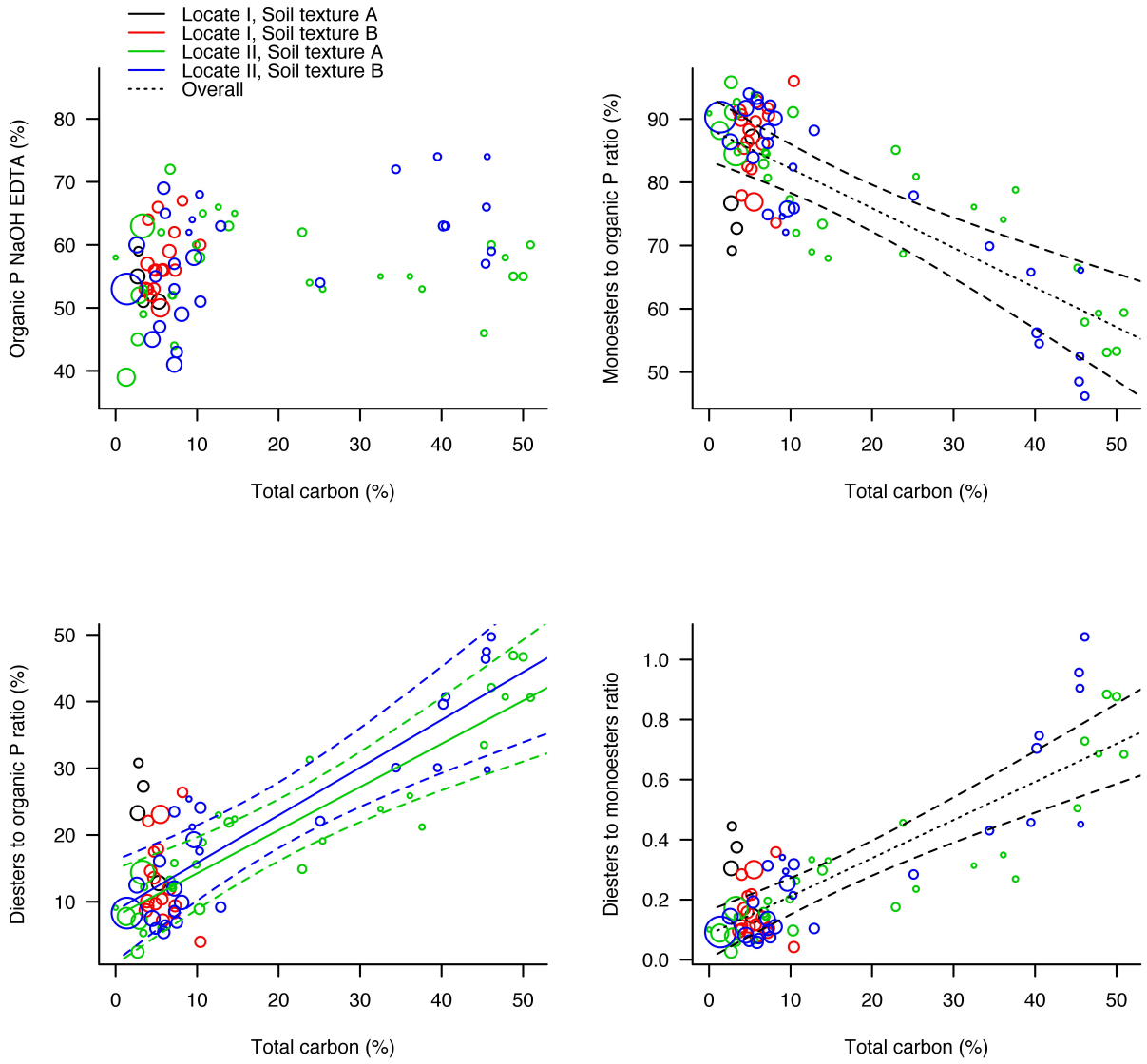

Figure 2. Soil carbon concentration influence on soil organic phosphorus dynamics on natural ecosystems. Locates were from (I) between and (II) below-above Capricorn and Cancer Tropics, while soil textures were (A) non-clayey soils (<35% clay) and (B) clayey soils (>35% clay). The point sizes were drawn proportional to the inverse of the weights (i.e., higher NaOH EDTA extraction are shown as larger points). Based on a mixed-effects model, the predicted responses with corresponding 95% confidence interval bounds were added to the plots.

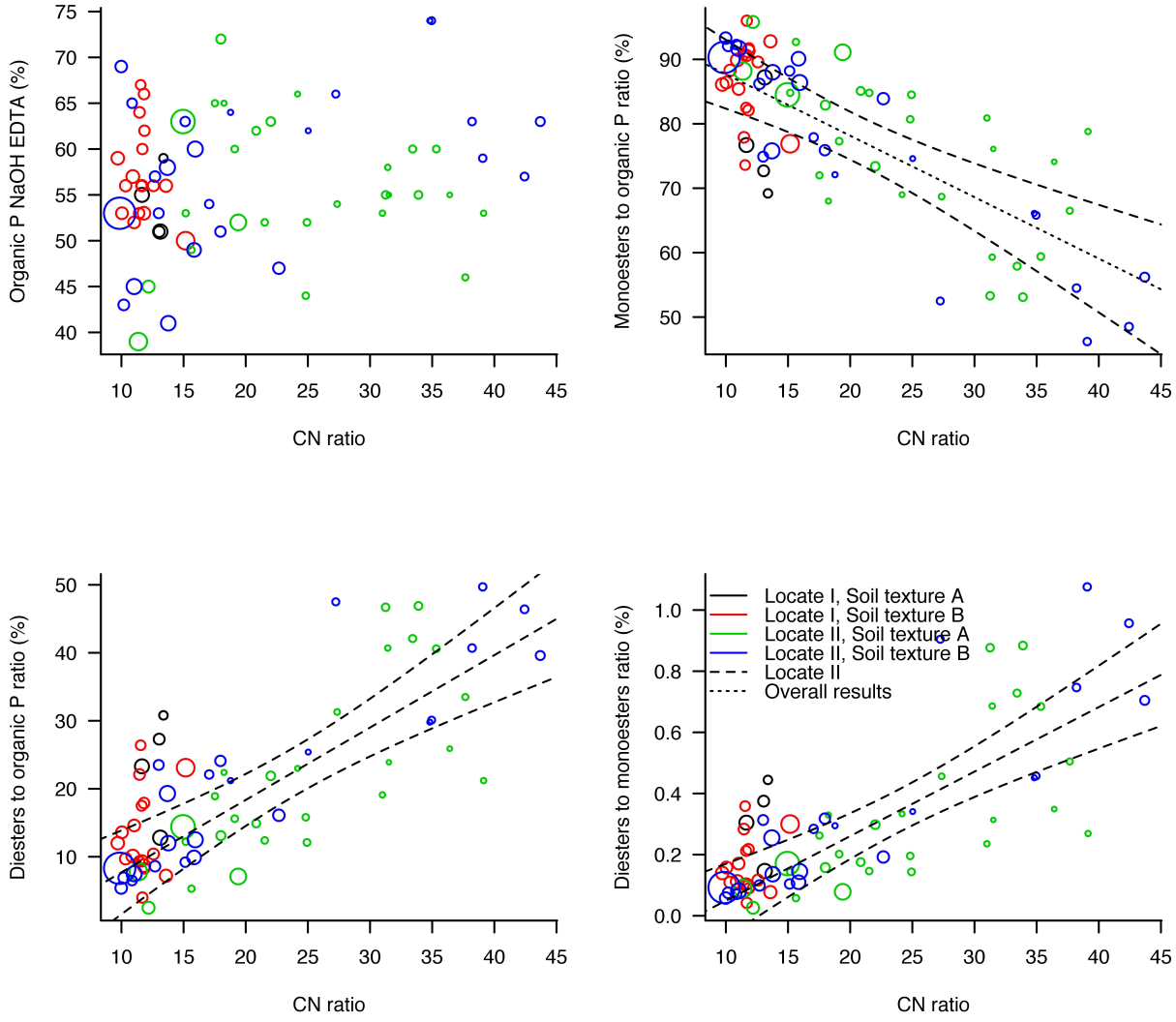

Figure 3. Soil carbon to nitrogen (CN) ratio influence on soil organic phosphorus dynamics on natural ecosystems. Locates were from (I) between and (II) below-above Capricorn and Cancer Tropics, while soil textures were (A) non-clayey soils (<35% clay) and (B) clayey soils (>35% clay). The point sizes were drawn proportional to the inverse of the weights (i.e., higher NaOH EDTA extraction are shown as larger points). Based on a mixed-effects model, the predicted responses with corresponding 95% confidence interval bounds were added to the plots.

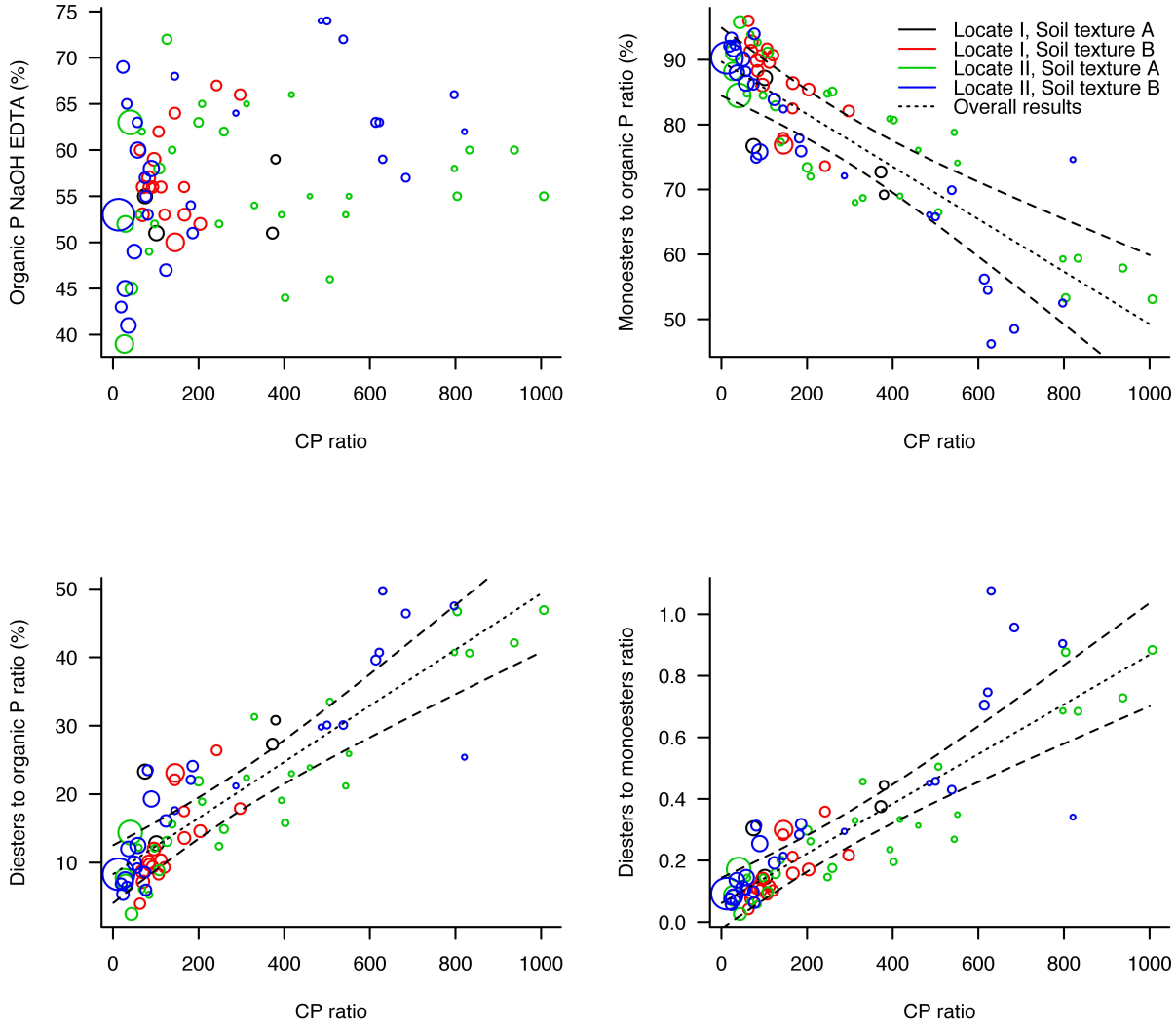

Figure 4. Soil carbon to phosphorus (CP) ratio influence on soil organic phosphorus dynamics on natural ecosystems. Locates were from (I) between and (II) below-above Capricorn and Cancer Tropics, while soil textures were (A) non-clayey soils (<35% clay) and (B) clayey soils (>35% clay). The point sizes were drawn proportional to the inverse of the weights (i.e., higher NaOH EDTA extraction are shown as larger points). Based on a mixed-effects model, the predicted responses with corresponding 95% confidence interval bounds were added to the plots.

Chapter 3

Soil texture and organic carbon fractions predicted from near-infrared spectroscopy and geostatistics [1]

[1] This manuscript is presented according to the Soil Science Society of America Journal style guidelines.

Soil texture and organic carbon fractions predicted from near-infrared spectroscopy and geostatistics

Highlights

- Near-infrared spectroscopy (NIRS) was appropriate to predict soil texture and carbon
- Both linear and non-linear multivariate models could be used for NIRS calibration
- Soil texture was predicted with greater precision than organic carbon fractions
- NIRS and geostatistics were a useful combination to assess spatial variation

ABSTRACT

Near-infrared spectroscopy (NIRS) and geostatistics are relatively unexplored tools that could reduce time, labor, and costs of soil analysis. Our objective was to efficiently determine lateral and vertical distributions of soil texture and soil organic carbon (SOC) fractions in an agroforestry system (7-ha field) on a Coastal Plain site in North Carolina. To predict selected properties from a large number of soil samples collected from this field, NIRS was calibrated against laboratory-determined properties. Support vector machines was a multivariate model that performed better than partial least squares to obtain greater precision with NIRS for all soil properties. To predict soil properties with precision across the field, geostatistical modeling with maximum likelihood and ordinary kriging was used. Combining the two modeling processes, root mean square error (RMSE) and RMSE relative to data set mean (% RMSE) were 67 g kg^{-1} for sand (9.3% RMSE), 34 g kg^{-1} for clay (22.7% RMSE), 1.63 g kg^{-1} for total organic C (26.7% RMSE), 0.67 g kg^{-1} for particulate organic C (36.1% RMSE), and 24 mg CO_2-C kg^{-1} 3 d^{-1} for the flush of CO_2 (29% RMSE). We conclude that the combination of NIRS and geostatistics produced acceptable errors, and therefore, could be used to predict

spatial distribution of soil texture and SOC fractions in this agroforestry system to allow efficient assessment of management changes with time and better predict small-scale input requirements.

Keywords: Environmental quality; Precision management; Spatial modeling.

INTRODUCTION

Soil organic carbon (SOC) and its fractions are important components for regulating environmental quality and yield potential of agricultural systems. Dynamics of SOC depend on climate, soil and landscape characteristics, and management. Better understanding of SOC dynamics at field-to-watershed scales will help improve soil management and its influence toward improving food security, mitigating greenhouse gas emissions, enhancing agricultural resilience, and conserving natural biodiversity. Fine-tuning management with geographic precision (e.g. precision application of fertilizer and pesticides) will be important to achieve sustainable intensification and development of sustainable working landscapes.

Within each crop growing season there is continuous variation in temperature, precipitation, solar radiation, humidity and other features of the weather, which are also the main causes of variation in plant productivity among growing seasons (Schueller, 2010). Beyond environmental conditions, inherent soil characteristics and variable agricultural practices can influence biologically sensitive soil properties, such as particulate organic C and mineralizable C and N. These soil properties often have greater magnitude of variation in space and time compared to soil texture or other physico-chemical properties (Aiken et al., 1991; Kreba et al., 2013). Moreover, within a

field scale in which the same agricultural practices are applied and climate varies little, inherent soil characteristics can impact organo-mineral associations through organic matter inputs, soil texture, phyllosilicate type, and oxyhydroxide concentrations. Therefore, understanding the relationship between these variables in space and time can support decision making for improved agricultural production and its consequences on environmental quality.

Precision management based on variable soil characteristics is often used to increase sustainability through variable-rate application of fertilizers, but precision management can also be used to design manure-application strategies, crop-pasture arrangements, successful intercropping, etc. Having tools to support implementation of precision agriculture could enable synergies to enhance production or to avoid environmental leakages. For example, using the flush of CO_2 to guide fertilizer-N supply could more accurately satisfy crop demand with exogenous input to balance profitability and environmental quality (Franzluebbers, 2016). In this case, greater management precision could impact all system components, including crop and pasture yield, greenhouse gas emissions, water and soil quality, and economic viability.

Investigations to analyze agroecosystem properties using visible-, near- and mid-infrared spectroscopy jointly with geostatistics (e.g. for soil properties, Odlare et al., 2005; Cobo et al., 2010; Bilgili et al., 2011; Conforti, et al., 2013; Shen et al., 2013; Steffens and Buddenbaum, 2013), can be done: (i) in-situ, (ii) with samples in the laboratory, or (iii) from images collected by satellite or unmanned aerial vehicle. Linking spectroscopy with geostatistics can be done by (i) processing spectral information by chemometrics and using predicted results in spatial analyses or (ii) through integration of

chemometric coefficients (e.g. partial least squares scores) to improve modeling of the geostatistical step. We explored only the first approach in this study.

Although some investigations have demonstrated the possibility to predict SOC fractions using visible-, near- and mid-infrared spectroscopy (Vasques et al., 2009; Viscarra-Rossel and Hicks, 2015), few studies have coupled this technique with geostatistics to predict total SOC concentration (Conforti, et al., 2013; Shen et al., 2013) and various SOC fractions (e.g. mineral-associated and readily mineralizable) in agricultural fields.

Our specific hypothesis was that soil texture and SOC fractions (total, particulate, and mineralizable) in an agroforestry system experiment on a Coastal Plain site in North Carolina could be estimated using near-infrared spectroscopy (NIRS) and geostatistics. A broader hypothesis was that agricultural productivity and environmental quality of fields and regions could become more balanced if geographic precision of soil properties is known, and subsequently managed appropriately.

Our objective was to determine vertical and lateral distribution of soil properties in a recently established agroforestry system on a Coastal Plain site in North Carolina. Specifically, using a combination of NIRS and geostatistics we wanted to characterize total, particulate, and mineralizable C fractions across a 7-ha field and at 0-5, 5-15, and 15-30 cm depths.

MATERIALS AND METHODS

Site Characteristics and Field Design

An agroforestry experiment was established in 2007 on a 7-ha field of alluvial bottomland near the Neuse River in Wayne County, North Carolina (35°22’ N 78°02’ W).

Soil properties were expected to vary due to alluvial deposition processes, which resulted in visual differences in water infiltration and plant growth. Soil was mapped as predominantly Coxville loam (fine, kaolinitic, thermic Typic Paleaquults) and Lakeland sand (thermic, coated Typic Quartzipsamments), but with minor contributions from Chewacla loam (fine-loamy, mixed, active, thermic Fluvaquentic Dystrudepts) and Leaf loam (fine, mixed, active, thermic Typic Albaquults). Soil pH varied from 5.9 to 6.6 (Franzluebbers et al., 2016). The study site has flooded several times in the recent past when the Neuse River rises beyond its limits, but not during soil sampling.

Design of the agroforestry experiment consisted of three tree species and two alley widths within each of five blocks (Cubbage et al., 2012). Trees selected were longleaf pine (*Pinus palustris* Mill.), loblolly pine (*Pinus taeda* L.), and cherrybark oak (*Quercus pagoda* Raf.), all native to the region and loblolly pine as most common commercial species in the region. Trees were planted in rows running east-west with three lines per row (diamond-shaped spacing of 1.8 m between trees in each line and 1.8 m between lines). Alleys between tree rows were 12 and 24 m wide and each block contained two sets of these alleys. Alleys were cropped with conventional management for soybean [*Glycine max* (L.) Merr.] in 2007, 2009, and 2011, for corn (*Zea mays* L.) in 2008, 2010, and 2012, and for annual ryegrass (*Lolium multiflorum* L.) in 2013/14.

Soil Collection

Soil was collected from 0-30 depth with a hydraulic probe (4-cm inside diam.) (Giddings Machine Company Inc.) mounted on a Polaris Ranger ® at 204 locations throughout the field on 24 April 2014 (immediately after the first cutting of ryegrass hay). Sampling was only in the herbaceous cropped area to serve as a baseline for long-term

assessment of the agroforestry site. At each of the 204 sites, one core was sectioned into three depth increments (0-5, 5-15, and 15-30 cm) and a second core within 0.5 m distance at 0-5-cm depth only was mixed with the other one, resulting in total soil volume sampled of 126 cm^3 for 0-5- and 5-15-cm depths and 188 cm^3 for 15-30-cm depth. The additional core was sampled to have enough volume for all laboratory analyses.

Sample Selection by Near-Infrared Spectroscopy

A total of 612 soil samples were dried (55 °C) and ball-milled before obtaining spectra via NIRS. Spectra were obtained with a scanning monochromator (Model 5000, Foss NIRSystems Inc., Silver Spring, MD). Each sample was the average of 32 scans and 16 reference scans before and after each sample. The SELECT algorithm with a minimum standardized Mahalanobis distance of 0.6 to explain ≥95% of total variance was used to determine which samples to calibrate against laboratory analyses (Shenk and Westerhaus, 1991). This resulted in 257 samples selected using a spectral treatment Gap-Segment, first derivative order and segment size 4 followed by Detrend.

Laboratory Analyses

Laboratory analyses were conducted on 257 of the 612 total samples. Near-infrared spectroscopy was used to predict soil characteristics of the 355 remaining samples (described in detail below). Laboratory analysis of total organic C, particulate organic C, the flush of CO_2 (as a proxy for mineralizable C), and soil clay and sand concentrations followed protocols similar to those presented in Franzluebbers et al. (2007). Samples were dried (55 °C, ≥3 d), weighed, and homogenized by passing through a sieve with 4.75-mm openings. Bulk density was determined from dry weight

and volume of the coring device. A subsample was ball-milled and analyzed for total organic C with a Leco Tru-Spec CN analyzer. Soil texture and particulate organic C were determined with dispersion of sieved soil using sodium pyrophosphate solution (0.1 mol L^{-1} $Na_4P_2O_7$). Following shaking overnight, the soil solution was poured into a glass cylinder and brought to 1-L volume with deionized water. Clay concentration was determined by the hydrometer method following thorough mixing in the cylinder and 5 h of settling time (Gee and Bauder, 1986). After that, soil dispersed in the sodium pyrophosphate solution was passed through a sieve with 0.053 mm openings and sand concentration was determined from the contents retained after passing the solution over the sieve. The sand fraction was transferred to a drying bottle with a stream of water and dried in an oven at 55 °C for 3 d, weighed, ball-milled, and analyzed for particulate organic C with a Leco Tru-Spec CN analyzer (Leco Corp., St. Joseph MI). Silt concentration was obtained by difference, subtracting clay and sand from 1000 g kg^{-1}. The flush of CO_2 following rewetting of dried soil (CMIN3) was determined from 50-g samples rewetted to 50% water-filled pore space in a sealed 1-L canning jar in the presence of 10 mL of 1 mol L^{-1} NaOH. Soil was incubated for 3 d at 25 °C. The alkali trap was titrated to a phenolphthalein endpoint with 1 mol L^{-1} HCl after precipitation of carbonate with 1.5 mol L^{-1} $BaCl_2$.

Near-Infrared Spectroscopy and Chemometrics

Following sample selection for calibration and validation, several spectral treatments were applied after obtaining absorbance (log R^{-1}) (R is reflectance) to remove physical information from the spectra and increase model robustness, accuracy, repeatability and reproducibility (Stevens and Ramirez-Lopez, 2015). Treatments included Savitzky-Golay

smoothing and derivative, gap-segment derivative, continuum-removal, detrend normalization, standard normal variate (SNV), block scaling, and sum of squares block weighting. Additional treatments included SNV transformation (Fearn, 2008) and detrend normalization after filtering (Savitzky-Golay and gap-segment).

Prior to calibration and validation, outliers were verified for each spectral treatment considering orthogonal distance and score distance. Orthogonal distance was between the true position of each data point and its projection in space of the first few principal components to explain ≥80% of total variance. Score distance was the projection of a sample to the center of all sample projections (Wehrens, 2011).

Calibration models were developed on a representative portion of data (75% training set), selected using the Kennard-Stone sampling algorithm to explain ≥95% of total variance and validated on remaining samples (25% test set). We used one linear and one non-linear multivariate regression model with different configurations to fit to the training set, which were subsequently fitted to the test set. The linear model was Partial Least Squares (PLS) and the non-linear model was Support Vector Machines (SVM). Configurations for each model were as follows. For PLS, three algorithms were tested, including kernel, SIMPLS, and classical orthogonal scores (Mevik and Wehrens, 2007). The number of principal components was determined by cross-validation (Varmuza and Filzmoser, 2009). For SVM, four kernels were tested, including linear, polynomial (second and third degrees), radial basis, and sigmoid (Karatzoglou et al., 2006). A common configuration that was tested in all models was with or without scaling function. By mean-centering, only spectra were scaled for PLS using The R Base Package (R Core Team, 2016), while spectra and predictor were scaled for SVM using the e1071 Package (Meyer et al., 2015).

Measured and predicted values of calibration and validation sets were evaluated for potential outliers using a linear model in the R Base Package (R Core Team, 2016). When potential outliers were found, samples were analyzed again in the laboratory to check whether there was analytical error. Verified data were reintroduced into the data set to repeat calibration and validation procedures.

Lowest root mean squared error (RMSE) of the 25% test set was used to determine selection of the best spectral treatment for each multivariate regression model (PLS and SVM). Selected regression models were then used to predict the remaining 355 samples. To compare among variables and other investigations, we calculated and reported %RMSE for both training and test sets (Steffens and Buddenbaum, 2013).

Geostatistics

Variogram models were constructed using classical estimator and maximum likelihood (ML) or restricted maximum likelihood (REML) methods (Diggle and Ribeiro Jr., 2007). Eight correlation functions were tested, including circular, cubic, gneiting, wave, exponential, spherical, matern, and powered exponential. For the latter two functions, additional smoothness or shape (κ) was used. κ ranged from 0.3-3.5 for matern and 0.3-1.75 for powered exponential correlation functions. Models were selected using maximized likelihood and Akaike Information Criteria. Anisotropy was checked by plotting data versus coordinates and empirical directional variograms for angles of 0^{o}, 45^{o}, 90^{o} and 135^{o}. Variograms were evaluated for pure nugget effect by envelopes built under the assumption of no spatial correlation, based on permutations of data across locations (Diggle and Ribeiro Jr., 2007).

Ordinary kriging was used to spatially interpolate data. Cross-validation using the leaving-one-out strategy was performed to geostatistics-predicted values on sample points after kriging and to measure errors generated from geostatistics versus NIRS predictions. Since values used as input in the geostatistical step were NIRS-predicted values, we performed an additional validation comparing original lab values with geostatistics-predicted values using RMSE.

Statistical Analyses

Statistical analyses were made using R Version 3.1.0 (© 2014 The R Foundation for Statistical Computing). Packages used were ChemometricsWithR (Wehrens, 2011), chemometrics (Varmuza and Filzmoser, 2009), prospectr (Stevens and Ramirez-Lopez, 2015), pls (Mevik and Wehrens, 2007), geoR (Ribeiro Jr. and Diggle, 2001), e1071 (Meyer et al., 2015) and stats (R Core Team, 2016).

RESULTS AND DISCUSSION

NIRS and Chemometrics

Spectral treatments and models used for NIRS-predicted soil properties are presented in Table 1. Among all spectral pre-treatments evaluated, Gap-Segment with first order derivative was most common with lowest error. Alternatives that fit better with other spectral treatments were the PLS model (i.e. Gap-Segment with second order derivative) for particulate organic C and PLS and SVM models (i.e. Gap-Segment followed by Detrend and Savitzky-Golay followed by Detrend, respectively) for the flush of CO_2 . Moreover, different configurations of multivariate regression models were used among soil properties (i.e., derivative orders, segment sizes, polynomial orders, and filter

length). Absorbance spectra of soil properties can be found in Supplemental Fig. S1. Scatter plots and RMSE of training and test sets are presented in Fig. 1. Descriptive statistics for measured and NIRS-predicted results are presented in Table 2.

Generally, PLS and SVM regression models produced similar errors (Fig. 1). However, SVM performed better than PLS based on lowest RMSE and %RMSE. Significant predictability was found for all soil properties, but according to lowest %RMSE, greatest predictability followed the order: sand > clay > total organic C > flush of CO_2 > particulate organic C.

Total organic C had %RMSE of 21.9% and 16.7% using PLS for training and test sets, respectively, and 17.2% and 14.4% using SVM. Viscarra-Rossel and Behrens (2010) found %RMSE values for SOC of 30%, while Vasques et al. (2009) obtained %RMSE of 2% for both training and test sets using log-transformed values of SOC. Influence of logarithmic transformation of total organic C on RMSE is discussed below together with results for particulate organic C and the flush of CO_2.

Particulate organic C had %RMSE of 56.1% and 32.3% using PLS for training and test sets, respectively, and 45.7% and 29.0% using SVM. For log-back-transformed particulate organic C in Australian soils, %RMSE was 81.1% (Viscarra-Rossel and Hicks, 2015).

The flush of CO_2 had %RMSE of 44.6% and 27.7% using PLS for training and test sets, respectively, and 18.8% and 26.6% using SVM. We modeled particulate organic C and the flush of CO_2 using natural log transformation, but RMSE was determined on back-transformed values. We verified that %RMSE was not proportional after back-transforming data and values increased considerably. For example, for the test set of the flush of CO_2, %RMSE changed from 7.7% to 27.7% using PLS and from 6.6% to 26.6%

using SVM. Vasques et al. (2009) used visible-NIRS to predict soil C mineralization and found %RMSE of 7.3% on log-transformed data. For cumulative (mg CO_2-C kg^{-1}) and basal respiration rate (mg CO_2-C kg^{-1} d^{-1}) of soil determined from 35-d incubations at 25 °C, Chang et al. (2001) found %RMSE of 72.8% and 52.3%, respectively.

For sand, %RMSE of training and test sets was 8.0% and 5.9% using PLS, respectively, and 6.6% and 5.1% using SVM (Fig. 1). For clay, %RMSE of training and test sets was 8.1% and 11.0% using PLS, respectively, and 8.1% and 8.5% using SVM. Predictability of clay and sand in this study was quite good compared with results of other studies. For clay determination in Australian soils, %RMSE was ~23% using SVM and PLS (Viscarra-Rossel and Behrens, 2010). Sand and clay fractions predicted by NIRS in Iowa and Minnesota soils had large %RMSE (41-135% for sand and 16-24% for clay) (Chang et al., 2005). In a glacial-till soil in Michigan, clay concentration derived from an on-the-go NIRS using PLS was predicted with moderate %RMSE of 28% (Shen et al., 2013).

Many factors could have led to differences in NIRS predictability between our and other studies, including instrumentation, modeling, sample preparation, and soil characteristics. Larger data sets from other studies could have created greater variation of spectral characteristics, resulting in a negative influence on the modeling process (i.e., increased error sizes) (Chang et al., 2001; Viscarra-Rossel and Behrens, 2010; Viscarra-Rossel and Hicks, 2015). Sample preparation could have also impacted prediction. We worked with oven-dried and ball-milled soil samples, while many other studies have used air-dried and 2-mm sieved soils samples. Dried and ball-milled soil samples reduces the light scattering effect (Gobrecht et al., 2014) and minimizes water interference on spectral reflectance. Moreover, spectral treatments (Gobrecht et al., 2014) and non-

linear multivariate models can be useful to reduce nonlinearities from the scattering effect. The on-the-go NIRS (Shen et al., 2013) can be subject to potentially greater interference from water and light scattering, but alternatives have been proposed to minimize these negative effects, such as using a normalized soil moisture index (Nocita et al., 2013).

Compared with the current routine use of NIRS for plant analyses, soil scientists might need to consider different spectral treatments and models to be able to predict unique soil properties. Some soil properties, such as total organic C, total N, moisture content, particle size, and aggregation, have a strong physical-theoretical basis for prediction with NIRS. If a variable is correlated with one or more of these properties, it may also be predicted by NIRS, even though that property does not respond directly to near-infrared light (Chang et al., 2001). This associative nature can have a great advantage for predicting SOC fractions, because they can be present in low concentration (e.g. the flush of CO_2) or be defined by a specific particle size-fraction (e.g. particulate organic C). Discovering differences in SOC fractions is complicated by their oft-dynamic nature and complex associations in the soil matrix. Strong associations were made previously for potentially mineralizable N with total organic C and total N and for cation-exchange capacity with clay and organic matter concentration (Chang et al., 2001).

Calibration of NIRS for universal prediction of soil properties is a challenge (Chang et al., 2001), because calibrating a variable in one soil may not be acceptable for another soil with different chemical, physical, and biological interactions that are defined by soil forming factors (climate, organisms, relief, parent material, and time) and management. Better prediction of clay and sand concentrations by NIRS than of SOC

fractions was likely due to greater separation of dominating physical characteristics (texture) from lower-concentration differences in chemical (SOC) characteristics. Detection of a range of molecules is possible, because NIRS radiation causes molecular bonds (H-C, H-N, and H-O) to vibrate (bending or stretching), thereby allowing soil to absorb light. However, many factors including soil matrix and environmental conditions (e.g. moisture) (Stenberg et al., 2010) can negatively impact the quality of NIRS spectra to detect weakly separated signals. Vasques et al. (2009) verified that different spectral treatment and multivariate models (including linear and non-linear models) were needed to predict ecologically relevant SOC fractions in a mixed-use landscape in Florida. When scientists already have a pretreatment and model chosen for a specific property in a particular soil, then classification methods (e.g., using orthogonal and score distances) can be used to check whether new samples would be similar enough to the calibration data set.

Geostatistics

Most soil properties (NIRS-predicted using SVM) had strong spatial dependence (nugget-to-sill ratio < 25%) (Fig. 2 and Table 3). Some exceptions occurred, in which in the nugget-to-sill ratio was 50% for total organic C at 15-30-cm depth and the nugget-to-sill ratio was 87% for particulate organic C at 0-30-cm depth. The flush of CO_2 at 0-30-cm depth and particulate organic C at 15-30-cm depth had no spatial dependence (i.e. pure-nugget effect).

Sand and clay concentrations had increasing practical range and sill as depth increased (Fig. 2 and Table 3). Total organic C had greatest practical range at the 5-15 cm depth, followed by the 0-5 cm and 15-30 cm depths. Partial sill of total organic C was

similar at the 0-5 cm and 5-15 cm depths, but lower at the 15-30 cm depth. Particulate organic C had greater partial sill and practical range at the 0-5 cm than the 5-15 cm depth. Flush of CO_2 had lower nugget, partial sill, and practical range at 5-15 cm and 15-30 cm depths.

Spatial description combined with knowledge about a phenomenon can improve our understanding of physical underlying mechanisms controlling spatial patterns. For example, soil pH of a forested area had greater short-range variability than from a grassland area, reflecting large heterogeneity of ground vegetation in the forest compared with uniformity in the grassland (Goovaerts, 1998). In our results (using SVM in the geostatistical models), soil texture had longer ranges than SOC fractions (Fig. 2 and Table 3), which could have been a result of long-term influence of soil formation factors on soil texture heterogeneity, while heterogeneity of SOC fractions might have been more dependent on shorter term management and vegetation. According to Allen et al. (2010), soil series, clay concentration, and site management can have a major influence on SOC heterogeneity at the community scale (20 m to km), while vegetation influences SOC heterogeneity at finer scales. Peigné et al. (2009) also suggested that management would promote more variability for sensitive soil biological properties than for physicochemical properties.

For particulate organic C and the flush of CO_2, shorter ranges were found as depth increased, suggesting they were possibly influenced by greater spatial heterogeneity promoted by hot spots in deeper layers. Lower sills observed for total, particulate, and mineralizable C fractions as depth increased (Fig. 2 and Table 3) were probably due to lower inputs of C and resultant lower C concentrations deeper in the soil profile (Supplemental Table S1).

The pure nugget effect for particulate organic C (15-30-cm depth) and for the flush of CO_2 (0-30-cm depth) was possibly due to scale of the spatial evaluation. Sampling sites separated by a minimum distance of 8.2 m may have exceeded the spatial dependence of these properties at 15-30-cm depth. Peigné et al. (2009) also suggested that the 10 x 10 m grid used in their study might have been too coarse to reveal spatial structure for mineralizable C and N.

For all soil properties, NIRS-predicted values using SVM were chosen for mapping, because SVM resulted in lowest errors (Fig. 3). Geostatistics-predicted soil properties had greater %RMSE (9.3% for sand, 22.7% for clay, 26.7% for total organic C, 36.1% for particulate organic C, and 29.0% for the flush of CO_2) than NIRS-predicted values alone using SVM. Descriptive statistics of measurements from this comparison are presented in Supplemental Table S2.

Efficacy of modeling soil properties when combining NIRS and geostatistics is questionable because of error size. Analyzing the association between laboratory-measured values and geostatistics-predicted values (using NIRS-predicted values as input), lowest %RMSE was found for sand (RMSE = 67 g kg^{-1} and %RMSE = 9.3%) and highest %RMSE was for particulate organic C (RMSE = 0.67 g kg^{-1} and %RMSE = 36.1%) (Fig. 3). One possibility to improve the calibration of particulate organic C (i.e. reduce error) could be calibrating NIRS spectra of the sand fraction itself instead of the bulk soil sample. Since the mineral-associated organic C was dominant in the bulk sample, it may have masked partriculate organic C detection in the bulk sample. An alternative might be to normalize data by particle size. Accuracy and stability of a model normalized by particle size significantly improved prediction of total soil N compared with a model using original spectral data (Xiaofei et al., 2015). Another alternative might be

using ancillary data to improve modeling of the geostatistical step (Goovaerts and Kerry, 2010). Covariates could be derived from other soil variables, remote-sensing images, or coefficients from the chemometrics approach.

CONCLUSIONS

Near-infrared spectroscopy (NIRS) was a useful tool to predict soil texture and soil organic carbon (SOC) fractions. Using chemometrics to calibrate NIRS, a support vector machine (SVM) model performed better than a partial least squares (PLS) model to predict soil texture (sand and clay) and SOC fractions (total, particulate, and mineralizable C). Geostatistics increased errors of soil properties compared to those obtained solely by NIRS prediction. Nonetheless, geostatistics was useful to explore spatial patterns (lateral and by depth) of soil texture and SOC fractions. Combining NIRS and geostatistics will be useful to determine future management influences on soil properties specifically in this agroforestry system, but also in neighboring fields and landscapes. This approach can be promoted for evaluation in other landscapes to increase sustainability of agroecosystems through field-specific precision management.

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Figure Captions

Figure 1. Calibration scatter-plots and root mean squared errors (RMSE) of soil properties measured in the laboratory versus predicted using near-infrared spectroscopy modeled with either Partial Least Squares or Support Vector Machines in multivariate regression. See Table 1 for pretreatments and model details. The RMSE of particulate organic C and the flush of CO_2 were determined on logarithmic back-transformed data.

Figure 2. Variograms of soil properties using near-infrared spectroscopy as input when modeled with Partial Least Squares (PLS) or Support Vector Machines (SVM) in multivariate regression. See Table 1 for multivariate regression model details and Table 3 for geostatistics details. Where a pure nugget effect was detected (particulate organic C at 15-30-cm depth and the flush of CO_2 at 0-30-cm depth) a confidence interval assuming no spatial correlation (envelopes) was shown instead of fitted line.

Figure 3. Validation scatter-plots and root mean squared errors (RMSE) of soil properties measured in the laboratory versus predicted by geostatistics using near-infrared spectroscopy as input when modeled with Partial Least Squares and Support Vector Machines in multivariate regression. Particulate organic C includes only 0-5- and 5-15-cm depths because of a pure-nugget effect observed at 15-30-cm depth, while all other soil properties have data at all three depths. See Table 1 for multivariate regression model details and Table 3 for geostatistics details.

Figure 4. Soil sand and clay concentration in an agroforestry system experiment on a Coastal Plain site in North Carolina.

Figure 5. Soil total organic C and particulate organic C in an agroforestry system experiment on a Coastal Plain site in North Carolina. Particulate organic C includes only 0-5- and 5-15-cm depths because of a pure-nugget effect observed at 15-30-cm depth.

Figure 6. The flush of CO_2 following rewetting of dried soil in an agroforestry system experiment on a Coastal Plain site in North Carolina. The flush of CO_2 includes only 0-5, 5-15-cm, and 15-30 cm depths because of a pure-nugget effect observed at 0-30-cm depth.

Supplemental Figure S1. Absorbance spectra based on quartiles of scans of soil properties. Absorbance spectra were pretreated according to conditions in Table 1 before soil properties were predicted from near-infrared spectroscopy with Partial Least Squares and Support Vector Machines in multivariate regression as input.

Table 1. Spectral treatments and models used to predict soil properties in an agroforestry system on a Coastal Plain site in North Carolina using laboratory-based near-infrared spectroscopy and multivariate regression models: Partial Least Squares (PLS) and Support Vector Machines (SVM).

Soil property	Model	Spectral pre-treatment †	Arguments ‡	Multivariate regression model details §
Sand	PLS	G-S	DO 1, SS 10	classical algorithm, scaled, 9 PC
Sand	SVM	G-S	DO 1, SS 10	kernel linear, scaled, 137 SV
Clay	PLS	G-S	DO 1, SS 1	kernel algorithm, scaled, 13 PC
Clay	SVM	G-S	DO 1, SS 10	kernel linear, scaled, 124 SV
Total organic C	PLS	G-S	DO 1, SS 10	classical algorithm, unscaled, 4 PC
Total organic C	SVM	G-S	DO 1, SS 10	kernel linear, scaled, 125 SV
Particulate organic C ¶	PLS	G-S	DO 2, SS 10	SIMPLS algorithm, unscaled, 3 PC
Particulate organic C ¶	SVM	G-S	DO 1, SS 10	kernel linear, scaled, 144 SV
Flush of CO_2 ¶, #	PLS	G-S + DT	DO 1, SS 5, FL 11, DT	SIMPLS algorithm, unscaled, 3 PC
Flush of CO_2 ¶, #	SVM	S-G + DT	DO 1, PO 1, FL 11, DT	kernel radial, scaled, 156 SV

† Savitzky-Golay (S-G), Gap-Segment (G-S), Detrend (DT). ‡ Absorbance was the spectral basis for all other pretreatments. Derivative orders (DO), segment sizes (SS), polynomial orders (PO), filter length (FL) and Detrend (DT). § For PLS three algorithms were tested: the kernel, SIMPLS and the classical orthogonal scores. For SVM, four kernels were tested: linear, polynomial (second and third degrees), radial basis and sigmoid. CP: number of principal components. SV: number of support vectors. For PLS only spectra was scaled and for SVM spectra and predictor were scaled. ¶ obtained with logarithmic transformation of data. # Flush of CO_2-C during 3 d following rewetting of dried soil.

Table 2. Descriptive statistics of soil properties in an agroforestry system on a Coastal Plain site in North Carolina, as measured or predicted by near-infrared spectroscopy (NIRS) and multivariate regression models using Partial Least Squares (PLS) and Support Vector Machines (SVM).

		Measured				NIRS-Predicted			
	n	Min.	Med.	Mean	Max.	Min.	Med.	Mean	Max.
Sand (g kg^{-1}) training set PLS	193	80	730	690	930	60	740	690	980
Sand (g kg^{-1}) test set PLS	64	600	810	790	870	560	800	780	920
Sand (g kg^{-1}) training set SVM	193	80	730	690	930	90	740	690	940
Sand (g kg^{-1}) test set SVM	64	600	810	790	870	580	810	790	910
Clay (g kg^{-1}) training set PLS	193	70	140	160	450	60	140	160	440
Clay (g kg^{-1}) test set PLS	64	80	100	110	180	70	100	110	190
Clay (g kg^{-1}) training set SVM	193	70	130	160	450	60	130	160	430
Clay (g kg^{-1}) test set SVM	64	80	100	110	270	80	100	110	280
TOC (g kg^{-1}) training set PLS	193	0.9	5.7	6.4	18.3	0.03	6.1	6.4	14.1
TOC (g kg^{-1}) test set PLS	64	1.7	5.2	5.4	12.7	1.3	5.6	5.6	11.6
TOC (g kg^{-1}) training set SVM	193	0.9	5.7	6.4	18.3	0.09	6.1	6.3	14.3
TOC (g kg^{-1}) test set SVM	64	1.7	5.2	5.4	12.7	0.4	5.2	5.4	11.3
POC (g kg^{-1}) training set PLS	193	0.06	0.84	1.25	5.22	0.08	0.81	1.13	5.49
POC (g kg^{-1}) test set PLS	64	0.24	1.07	1.24	3.64	0.24	1.06	1.21	3.34
POC (g kg^{-1}) training set SVM	193	0.06	0.95	1.25	5.22	0.10	0.89	1.20	6.65
POC (g kg^{-1}) test set SVM	64	0.19	0.99	1.24	3.62	0.15	0.93	1.17	3.56
CMIN3 (mg kg^{-1}) training set PLS	193	8	70	83	245	13	58	81	314
CMIN3 (mg kg^{-1}) test set PLS	64	13	72	79	257	16	62	74	206
CMIN3 (mg kg^{-1}) training set SVM	193	8	69	80	245	13	67	77	207
CMIN3 (mg kg^{-1}) test set SVM	64	12	78	90	257	16	79	85	192

Min.: minimum. Med.: median. Max.: maximum. TOC: Total organic C. POC: Particulate organic C. CMIN3: Carbon mineralization in 3 days determined from the flush of CO_2-C following rewetting of dried soil.

Table 3. Geostatistics models used to interpolate soil properties in an agroforestry system on a Coastal Plain site in North Carolina using laboratory-based near-infrared spectroscopy as input when modeled with Partial Least Squares (PLS) and Support Vector Machines (SVM) in multivariate regression.

Variable (NIRS)	Depth	Variogram model †	Parameter estimator ‡	Nugget	Partial sill	Practical range	MLH §	AIC ¶	C-V error #
Sand (PLS)	0-5 cm	exp	REML	30.48	28044	449	-1127	2263	0.029
	5-15 cm	sph	REML	612.1	27466	250	-1140	2287	-0.219
	15-30 cm	exp	REML	2393	36293	449	-1204	2417	-0.002
	0-30 cm	exp	ML	43.3	33284	482	-1147	2301	0.062
Sand (SVM)	0-5 cm	circular	REML	0	22828	189	-1107	2221	0.127
	5-15 cm	sph	REML	0	38406	349	-1116	2240	-0.219
	15-30 cm	exp	REML	2318	46678	582	-1203	2415	-0.051
	0-30 cm	exp	ML	138	60524	946	-1145	2298	0.105
Clay (PLS)	0-5 cm	circular	REML	0	4164	189	-934	1876	0.127
	5-15 cm	circular	REML	193	5947	241	-982.8	1974	0.039
	15-30 cm	PE (κ=0.75)	REML	0	49162	6533	-1069	2147	-0.233
	0-30 cm	exp	ML	0	10917	620	-1007	2022	0.079
Clay (SVM)	0-5 cm	circular	REML	0	4041	189	-931	1870	0.107
	5-15 cm	circular	REML	127	6286	241	-977	1961	<0.001
	15-30 cm	PE (κ=0.75)	REML	0	42354	5635	-1065	2139	-0.232
	0-30 cm	exp	ML	0	10547	607	-1005	2019	0.078
TOC †† (PLS)	0-5 cm	sph	REML	0	6.7	80	-384	776	0.019
	5-15 cm	matern (κ=0.75)	REML	0	6.2	98	-380	782	0.016
	15-30 cm	matern (κ=3.5)	REML	1.4	4.0	54.4	-418	843	0.009
	0-30 cm	matern (κ=1.0)	REML	1.8	104	79.3	-675	1358	0.057
TOC †† (SVM)	0-5 cm	matern (κ=0.75)	REML	0.22	8.2	146	-378	764	0.024
	5-15 cm	exp	REML	0	7.8	203	-371	750	0.010
	15-30 cm	matern (κ=2.5)	REML	1.8	3.7	86	-406	820	0.010
	0-30 cm	matern (κ=1.0)	REML	11	95	115	-665	1339	0.050
POC ‡‡ (PLS)	0-5 cm	circular	REML	0.22	0.72	73	-228	464	0.001
	5-15 cm	matern (κ=2.5)	REML	0	0.26	36	-117	241	0.007
	15-30 cm	pure nugget	-	0.06	-	-	-	-	-
	0-30 cm	matern (κ=0.75)	REML	0	3.0	63	-347	716	0.014
POC ‡‡ (SVM)	0-5 cm	PE (κ=0.75)	REML	0.20	0.97	324	-237	483	0.003
	5-15 cm	PE (κ=0.75)	REML	0	0.16	76	-77	161	0.002
	15-30 cm	pure nugget	-	0.05	-	-	-	-	-
	0-30 cm	cubic	REML	0.89	1.02	82	-323	653	0.004

Continue

Table 3. Continuation.

CMIN3 §§ (PLS)	0-5 cm	sph	REML	313	4798	124	-1041	2089	0.296
	5-15 cm	circular	REML	217	674	93	-913	1835	0.075
	15-30 cm	exp	REML	1.2	150	76	-750	1509	0.083
	0-30 cm	pure nugget	-	114	-	-	-	-	-
CMIN3 §§ (SVM)	0-5 cm	circular	REML	114	1962	128	-931	1871	0.253
	5-15 cm	sph	REML	189	741	100	-916	1840	0.107
	15-30 cm	matern (κ=0.3)	REML	20	192	64	-782	1573	0.088
	0-30 cm	pure nugget	-	94	-	-	-	-	-

† exp: exponential, sph: spherical, PE: powered exponential, and κ: additional smoothness or shape parameters.. ‡ ML: maximum likelihood. REML: restricted maximum likelihood. § MLH: maximized likelihood. ¶ AIC: Akaike Information Criteria. # C-V error: leaving-one-out kriging cross-validation error (i.e., mean of NIRS-predicted minus geostatistics-predicted values). †† TOC: Total organic C. ‡‡ POC: Particulate organic C. §§ CMIN3: Carbon mineralization in 3 d as determined by the flush of CO_2-C following rewetting of dried soil.

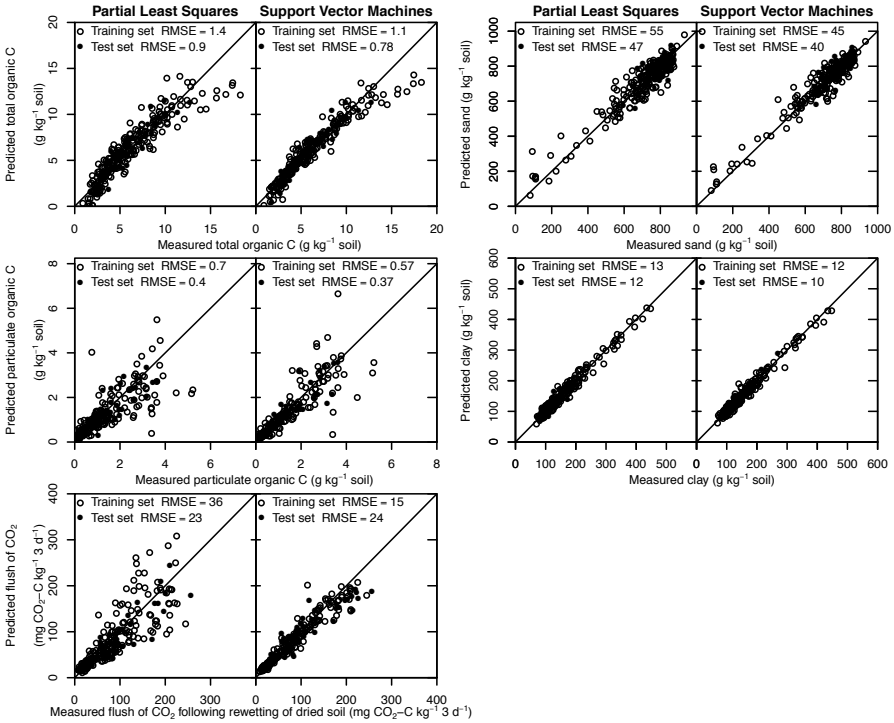

Partial Least Squares
Support Vector Machines
Predicted total organic C (g kg⁻¹ soil)
Training set RMSE = 1.4
Test set RMSE = 0.9
Training set RMSE = 1.1
Test set RMSE = 0.78
Measured total organic C (g kg⁻¹ soil)
Predicted sand (g kg⁻¹ soil)
Training set RMSE = 55
Test set RMSE = 47
Training set RMSE = 45
Test set RMSE = 40
Measured sand (g kg⁻¹ soil)
Predicted particulate organic C (g kg⁻¹ soil)
Training set RMSE = 0.7
Test set RMSE = 0.4
Training set RMSE = 0.57
Test set RMSE = 0.37
Measured particulate organic C (g kg⁻¹ soil)
Predicted clay (g kg⁻¹ soil)
Training set RMSE = 13
Test set RMSE = 12
Training set RMSE = 12
Test set RMSE = 10
Measured clay (g kg⁻¹ soil)
Predicted flush of CO2 (mg CO2–C kg⁻¹ 3 d⁻¹)
Training set RMSE = 36
Test set RMSE = 23
Training set RMSE = 15
Test set RMSE = 24
Measured flush of CO2 following rewetting of dried soil (mg CO2–C kg⁻¹ 3 d⁻¹)

Figure 1.

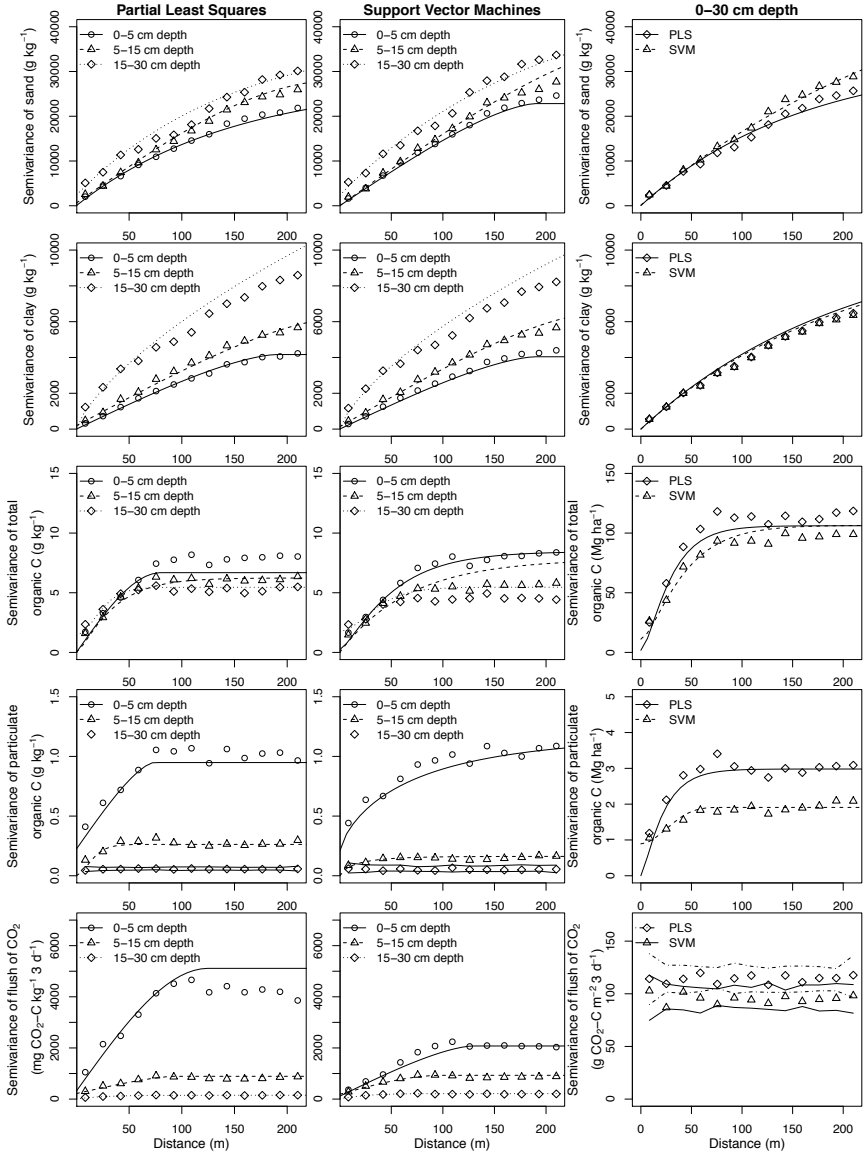

Partial Least Squares
Support Vector Machines
0–30 cm depth
0–5 cm depth
5–15 cm depth
15–30 cm depth
PLS
SVM
Semivariance of sand (g kg⁻¹)
Semivariance of clay (g kg⁻¹)
Semivariance of total organic C (g kg⁻¹)
Semivariance of total organic C (Mg ha⁻¹)
Semivariance of particulate organic C (g kg⁻¹)
Semivariance of particulate organic C (Mg ha⁻¹)
Semivariance of flush of CO2 (mg CO2–C kg⁻¹ 3 d⁻¹)
Semivariance of flush of CO2 (g CO2–C m⁻² 3 d⁻¹)
Distance (m)

Figure 2.

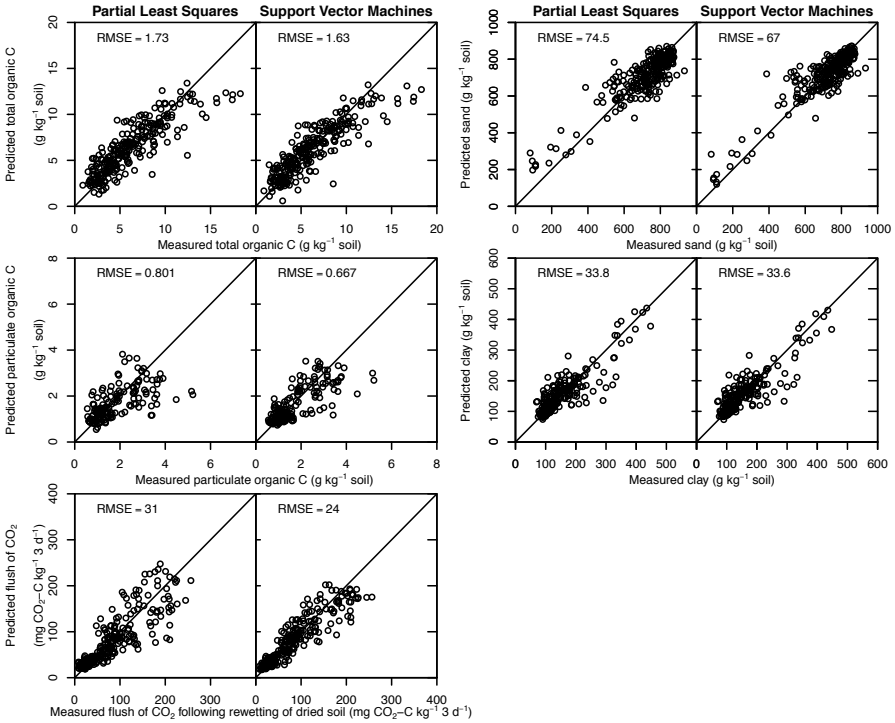

Partial Least Squares
Support Vector Machines
RMSE = 1.73
RMSE = 1.63
Predicted total organic C (g kg^{-1} soil)
Measured total organic C (g kg^{-1} soil)
RMSE = 74.5
RMSE = 67
Predicted sand (g kg^{-1} soil)
Measured sand (g kg^{-1} soil)
RMSE = 0.801
RMSE = 0.667
Predicted particulate organic C (g kg^{-1} soil)
Measured particulate organic C (g kg^{-1} soil)
RMSE = 33.8
RMSE = 33.6
Predicted clay (g kg^{-1} soil)
Measured clay (g kg^{-1} soil)
RMSE = 31
RMSE = 24
Predicted flush of CO_2 (mg CO_2–C kg^{-1} 3 d^{-1})
Measured flush of CO_2 following rewetting of dried soil (mg CO_2–C kg^{-1} 3 d^{-1})

Figure 3.

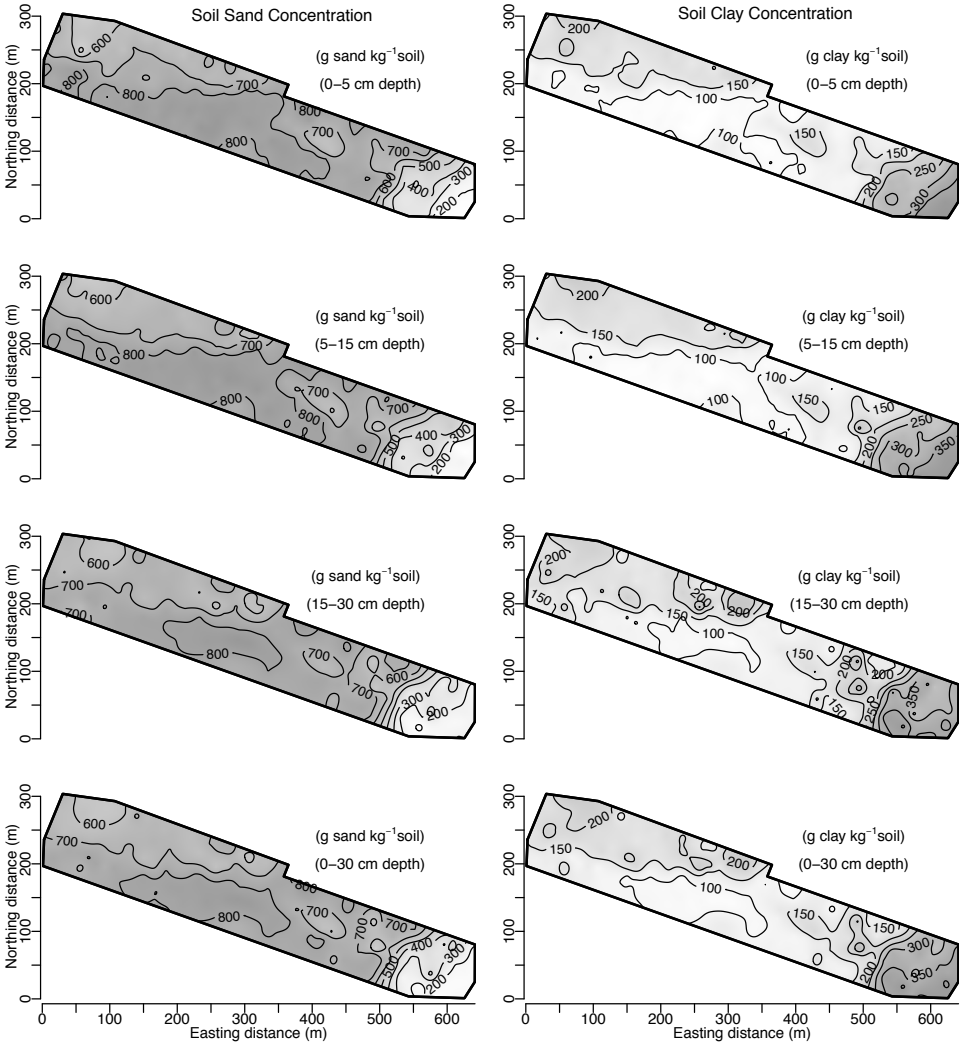

Soil Sand Concentration
(g sand kg^{-1}soil)
(0–5 cm depth)
Soil Clay Concentration
(g clay kg^{-1}soil)
(0–5 cm depth)
(g sand kg^{-1}soil)
(5–15 cm depth)
(g clay kg^{-1}soil)
(5–15 cm depth)
(g sand kg^{-1}soil)
(15–30 cm depth)
(g clay kg^{-1}soil)
(15–30 cm depth)
(g sand kg^{-1}soil)
(0–30 cm depth)
(g clay kg^{-1}soil)
(0–30 cm depth)
Northing distance (m)
Easting distance (m)

Figure 4.

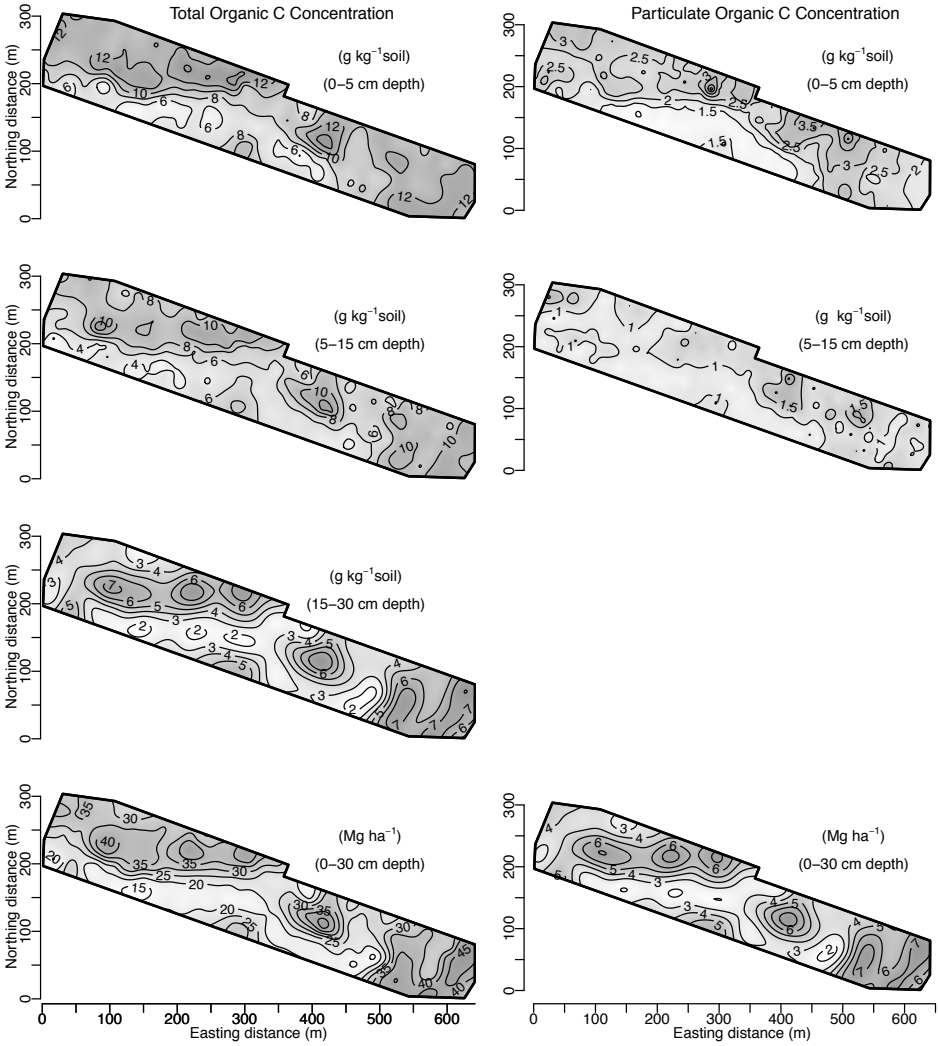

Total Organic C Concentration
Particulate Organic C Concentration
(g kg^{-1}soil)
(0–5 cm depth)
(5–15 cm depth)
(15–30 cm depth)
(Mg ha^{-1})
(0–30 cm depth)
Northing distance (m)
Easting distance (m)

Figure 5.

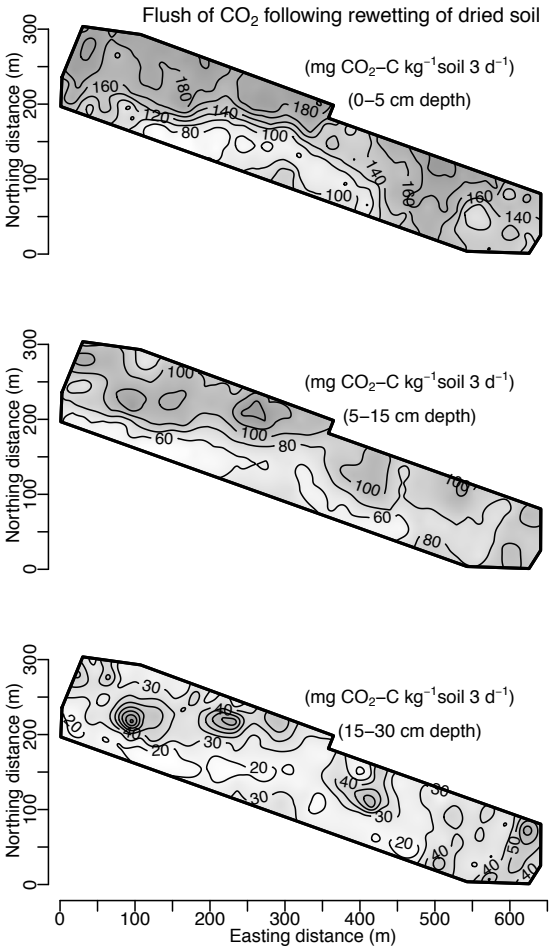

Flush of CO_2 following rewetting of dried soil
(mg CO_2–C kg^{-1}soil 3 d^{-1})
(0–5 cm depth)
(5–15 cm depth)
(15–30 cm depth)
Northing distance (m)
Easting distance (m)
0
100
200
300
400
500
600

Figure 6.

Supplemental Information

Supplemental Table S1. Descriptive statistics of soil properties in an emerging agroforestry system experiment on a Coastal Plain site in North Carolina predicted by laboratory-based near-infrared spectroscopy (NIRS) and multivariate regression models using Partial Least Squares (PLS) and Support Vector Machines (SVM) and geostatistics based on NIRS results as input.

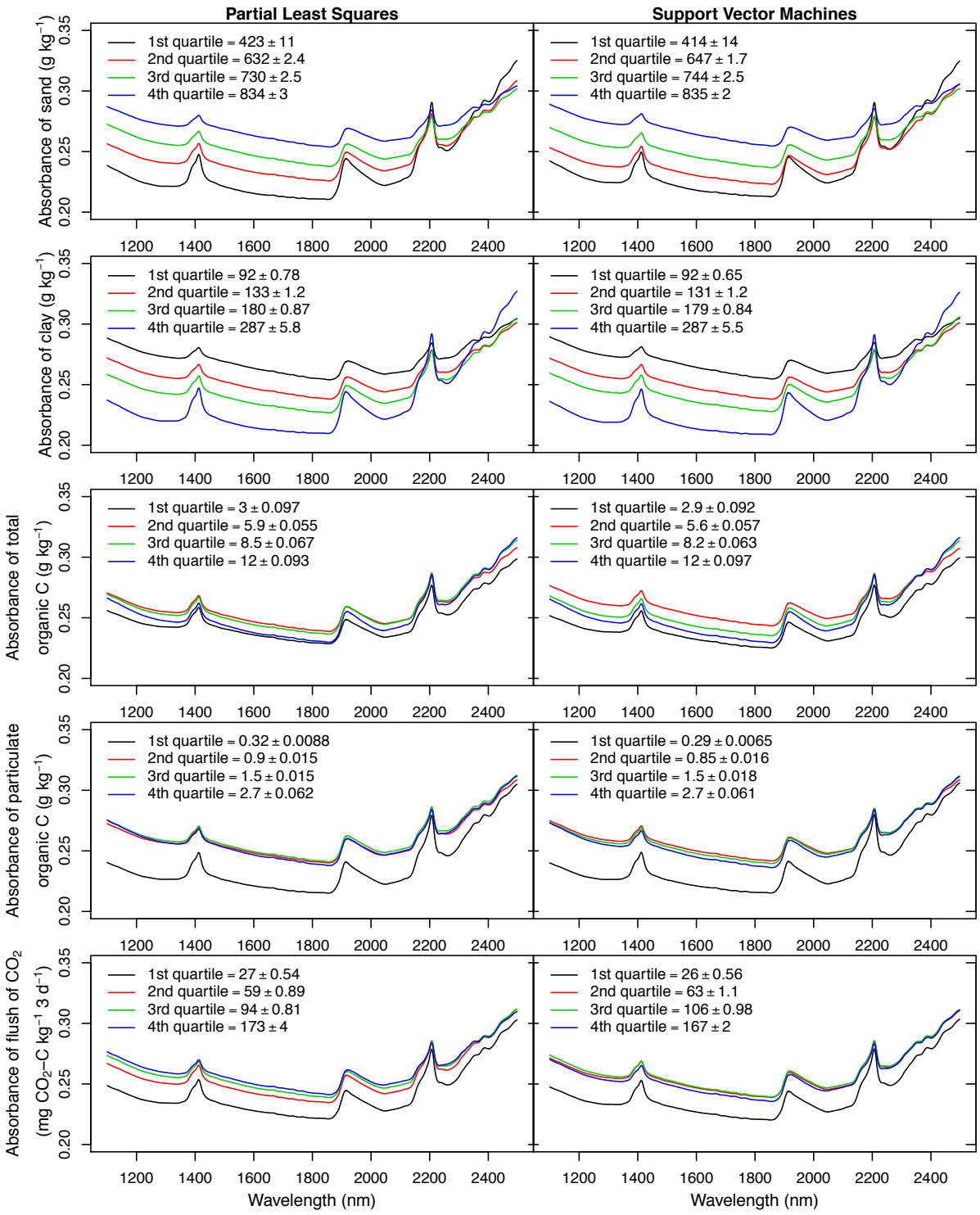
Variable	Model	Depth		NIRS-predicted				Geostatistics-predicted			
			n	Min.	Med.	Mean	Max.	Min.	Med.	Mean	Max.
Sand ($g\ kg^{-1}$)	PLS	0-5 cm	204	206	735	690	935	212	725	690	869
		5-15 cm	204	139	720	682	904	200	723	682	870
		15-30 cm	204	153	677	628	1050	197	666	627	860
Sand ($g\ kg^{-1}$)	SVM	0-5 cm	204	152	745	694	894	156	744	694	884
		5-15 cm	204	105	729	680	885	126	725	680	872
		15-30 cm	204	33	699	641	942	120	694	641	841
Clay ($g\ kg^{-1}$)	PLS	0-5 cm	204	76	133	155	397	82	136	154	387
		5-15 cm	204	66	136	159	414	74	138	159	389
		15-30 cm	204	59	158	190	473	87	167	190	437
Clay ($g\ kg^{-1}$)	SVM	0-5 cm	204	80	131	154	392	82	134	154	391
		5-15 cm	204	70	134	158	414	73	139	158	391
		15-30 cm	204	61	158	189	465	90	169	189	430
TOC ($g\ kg^{-1}$)	PLS	0-5 cm	204	3.5	10.3	9.8	14.6	5.1	10.4	9.8	13.7
		5-15 cm	204	1.8	7.3	7.4	13.9	3.4	7.5	7.4	12.0
		15-30 cm	204	0.0	4.1	4.4	11.0	1.3	4.3	4.4	8.8
TOC ($g\ kg^{-1}$)	SVM	0-5 cm	204	4.0	10.0	9.6	14.7	4.6	10.1	9.6	14.0
		5-15 cm	204	2.8	7.1	7.3	13.2	3.6	7.3	7.3	12.4
		15-30 cm	204	-1.8	3.7	4.1	12.1	0.4	3.8	4.1	8.8
POC ($g\ kg^{-1}$)	PLS	0-5 cm	204	0.74	2.11	2.24	5.49	0.95	2.18	2.23	3.83
		5-15 cm	204	0.44	1.09	1.22	4.03	0.54	1.15	1.21	2.57
		15-30 cm	204	0.08	0.39	0.42	2.01	-	-	0.42	-
POC ($g\ kg^{-1}$)	SVM	0-5 cm	204	0.75	2.19	2.32	6.65	1.06	2.38	2.32	3.69
		5-15 cm	204	0.38	1.00	1.08	2.72	0.73	1.03	1.08	1.88
		15-30 cm	204	0.08	0.33	0.37	3.01	-	-	0.37	-
CMIN3 ($mg\ kg^{-1}$)	PLS	0-5 cm	204	44	133	145	314	57	143	144	248
		5-15 cm	204	29	70	75	165	39	71	75	126
		15-30 cm	204	13	31	33	76	19	32	33	58
CMIN3 ($mg\ kg^{-1}$)	SVM	0-5 cm	204	57	148	145	220	68	147	145	202
		5-15 cm	204	36	86	87	171	46	86	87	140
		15-30 cm	204	13	30	33	100	15	32	33	71

Min.: minimum. Med.: median. Max.: maximum. TOC: Total organic C. POC: Particulate organic C. CMIN3: Carbon mineralization in 3 days determined from the flush of CO_2-C following rewetting of dried soil.

Supplemental Table S2. Descriptive statistics of soil properties in an emerging agroforestry system experiment on a Coastal Plain site in North Carolina from laboratory-measured or geostatistics-predicted values using near-infrared spectroscopy with Partial Least Squares (PLS) and Support Vector Machines (SVM) in multivariate regression as input.

Variable	Model			Laboratory Measured				Geostatistics Predicted			
		Depth	n	Min.	Med.	Mean	Max.	Min.	Med.	Mean	Max.
Sand ($g\ kg^{-1}$)	PLS	0-5 cm	85	194	795	763	871	322	789	749	864
		5-15 cm	66	337	750	726	872	389	764	739	870
		15-30 cm	106	81	717	666	933	197	678	648	840
Sand ($g\ kg^{-1}$)	SVM	0-5 cm	85	194	795	763	871	290	789	757	871
		5-15 cm	66	337	757	729	872	410	762	741	872
		15-30 cm	106	81	720	668	933	120	705	663	841
Clay ($g\ kg^{-1}$)	PLS	0-5 cm	85	83	113	128	351	82	115	128	322
		5-15 cm	66	75	110	130	325	74	120	132	274
		15-30 cm	106	70	148	175	448	87	163	179	437
Clay ($g\ kg^{-1}$)	SVM	0-5 cm	85	83	113	129	351	84	113	127	324
		5-15 cm	66	75	110	130	325	73	118	131	274
		15-30 cm	106	70	148	175	448	90	160	179	430
TOC ($g\ kg^{-1}$)	PLS	0-5 cm	85	4.2	8.5	8.8	18.3	5.1	8.8	8.8	13.4
		5-15 cm	66	3.6	6.1	6.7	14.5	3.7	6.7	6.7	10.8
		15-30 cm	106	0.9	3.3	3.7	12.5	1.3	3.8	4.1	7.6
TOC ($g\ kg^{-1}$)	SVM	0-5 cm	85	4.2	8.5	8.8	18.3	4.6	8.6	8.5	13.2
		5-15 cm	66	3.6	6.1	6.7	14.5	3.6	6.6	6.7	9.9
		15-30 cm	106	0.9	3.3	3.7	12.5	0.59	3.54	3.82	8.20
POC ($g\ kg^{-1}$)	PLS	0-5 cm	85	0.83	2.21	2.33	5.22	1.01	2.06	2.10	3.82
		5-15 cm	66	0.56	1.08	1.23	3.42	0.54	1.11	1.20	2.35
		15-30 cm	106	0.06	0.31	0.39	3.40	-	-	-	-
POC ($g\ kg^{-1}$)	SVM	0-5 cm	85	0.83	2.21	2.33	5.22	1.17	2.26	2.24	3.52
		5-15 cm	66	0.56	1.08	1.23	3.42	0.73	1.05	1.08	1.88
		15-30 cm	106	0.06	0.31	0.39	3.40	-	-	-	-
CMIN3 ($mg\ kg^{-1}$)	PLS	0-5 cm	85	48	141	143	257	57	142	141	248
		5-15 cm	66	50	85	88	139	45	77	76	115
		15-30 cm	106	8	28	30	73	19	32	32	51
CMIN3 ($mg\ kg^{-1}$)	SVM	0-5 cm	85	48	141	143	257	75	143	137	202
		5-15 cm	66	50	86	90	160	52	85	85	126
		15-30 cm	106	8	27	30	73	17	31	32	63

Min.: minimum. Med.: median. Max.: maximum. TC: Total organic C. POC: Particulate organic C. CMIN3: Carbon mineralization in 3 days determined from the flush of CO_2-C following rewetting of dried soil.



Supplemental Figure S1. Absorbance spectra based on quartiles of scans of soil properties. Absorbance spectra were pretreated according to conditions in Table 1 before soil properties were predicted from near-infrared spectroscopy with Partial Least Squares and Support Vector Machines in multivariate regression as input.

5. Final considerations

Here I will expose some thoughts to support other scientists and students that are willing to give sequence on the studies presented in this thesis.

In our first chapter we investigated the soil phosphorus composition only, but I believe many other aspects related to phosphorus cycling can still be explored. In addition to the effect of grazing livestock on soil phosphorus composition, livestock can impact plant dynamics and diversity of soil biological functioning, which can feed-back on the plant community and eventually on animal production. Therefore, the resultant feedbacks of the altered soil phosphorus composition could be explored. This would enable a better understanding of how soil phosphorus composition impacts both plant and animal performances as well as soil macro- and micro-organisms composition and activity (e.g., enzymatic activity, microbial biomass, and diversity). Since nutrients in cover crop forages were ingested by animals, passed through their digestive tract, and deposited as feces (predominantly) on soil, rapid transformation of organic phosphorus forms to orthophosphate can lead to immediate nourishment of subsequent crops. This may be dependent on feces deposition pattern on the pasture and feces decomposition rate, as influenced by temperature and rainfall, and importantly, macro- and meso-organisms interaction with the deposited dung. Moreover the different vegetal components of agricultural systems, such as the crop species, may have an impact on the phosphorus cycling through their different chemical composition and decomposition rates. Therefore, estimating both the vegetal residues and dung degradation rates (using proper litter bags, with proper openings size, e.g., Sitters et al., 2013), and evaluating residues and dung distribution, as affected by environmental factors and organisms movement (using single- or dual-labelling phosphorus, e.g., Noack et al., 2014), could

contribute to advance understanding of soil phosphorus dynamics on integrated crop-livestock systems.

Based on our second chapter, which dealt with research synthesis and meta-regression analysis, I observed some aspects that can improve future meta-analysis, to enable a better understanding of soil phosphorus dynamics on terrestrial ecosystems. More native vegetation results are required from locates between Capricorn and Cancer Tropics, on a greater soil carbon concentration range, for both clayey and non-clayey soils. This can be more easily achieved analyzing tropical high-lands soils. Also more results are required from alkaline soils of both locates (between and below-above Capricorn and Cancer Tropics). I also strongly recommend authors to provide results of all soil organic P species rather than only functional groups (e.g., as supplementary tables), even when species concentrations are low (and describe when species were not detected), what may enable future meta-analysis to avoid confounding effects of organic phosphorus species inside functional groups. Finally, variances or standard errors of soils with distinctive properties must be determined. Then, as stated by Stewart (2010), future meta-analysis can use the different amounts of information that studies of different sizes and different quality present.

Regarding the third chapter, an interesting matter to be studied is how soil texture affects soil organic carbon stabilization. Stabilizing soil organic carbon can help diminish climate change and its impacts on ecosystems. Soil texture is known to affect soil organic carbon concentration and soil microbial activity (i.e., mineralizable C), but clay mineralogy may affect these relationships. Soil organic matter stabilization can occur through a number of processes, including intrinsic chemical recalcitrance and quantity of C inputs, specific interactions with mineral surfaces and metal ions (organo-mineral

associations), and spatial inaccessibility to microbial decomposition due to physical occlusion within soil aggregates (Lützow et al., 2006; Carrington et al., 2012; Barré et al., 2014). Studies can be done by characterizing total, mineral-associated, and mineralizable C fractions along the gradient of soil texture. Samples can also be analyzed for soil texture, clay mineralogy, specific surface area, oxalate-extractable Al and Fe to estimate short-range-ordered (i.e., poorly crystalline) oxyhydroxides. Chemical composition of minerals in the organo-mineral interface region is highly complex, requiring detailed information about the chemical species present on mineral surfaces (Solomon et al., 2012).

Even the third chapter explored only carbon fractions, I believe it has an enormous potential to support future studies seeking to better understand nitrogen dynamics on agroecosystems. Using mineralizable C from the flush of CO_2 following rewetting of dried soil as a proxy to guide N fertilizer supply still requires calibrations at the field level (Franzluebbers, 2016), but strong relationships have been found between the flush of CO_2 and N availability in controlled laboratory incubations (Franzluebbers and Brock, 2007; Franzluebbers and Stuedemann, 2008) and semi- controlled greenhouse conditions (Franzluebbers, 2016). Short-term seasonal changes of the active mineralizable pool can be expected to occur, depending upon crop sequence, tillage and fertilization regimes (Franzluebbers et al., 1994), and organic replenishment of mineralizable pools during the growing season must be taken into account in a modeling process to predict N mineralization (Dessureault-Rompré et al., 2013). Therefore, knowledge of soil organic matter dynamics jointly with precision agriculture techniques can help contribute to meet food security (Gebbers and Adamchuk, 2010) for

a human population that seems likely to continue growing this century (Gerland et al., 2014).

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